ANTIBACTERIAL AGENTS & METHODS

TECHNICAL FIELD

The invention relates to means for carrying out conjugation between bacteria, and in particular the invention relates to carrier bacteria comprising antimicrobial agents and methods of use. A carrier bacterium is capable of conjugative transfer of DNA encoding the agent to a target cell.

The invention further relates to growth or feed conversion ratio (FCR) promotion in animals. The invention further relates to killing *Salmonella* or inhibiting the growth or proliferation of *Salmonella*. The invention further relates to killing *Pseudomonas* or inhibiting the growth or proliferation of *Pseudomonas*, such as useful for promoting growth, dry weight, wet weight or crop production of plants.

BACKGROUND

DNA sequences controlling extra-chromosomal replication (ori) and transfer (tra) are distinct from one another; i.e., a replication sequence generally does not control plasmid transfer, or vice- versa. Replication and transfer are both complex molecular processes that make use of both plasmid- and host-encoded functions. Bacterial conjugation is the unidirectional and horizontal transmission of genetic information from one bacterium to another. The genetic material transferred may be a plasmid or it may be part of a chromosome. Bacterial cells possessing a conjugative plasmid contain a surface structure (the sex pilus) that is involved in the coupling of donor and recipient cells, and the transfer of the genetic information. Conjugation involves contact between cells, and the transfer of genetic traits can be mediated by many plasmids. Among all natural transfer mechanisms, conjugation is the most efficient. For example, F plasmid of *E. coli*, pCF10 plasmid of *Enterococcus faecalis* and pXO16 plasmid of *Bacillus thuringiensis* employ different mechanisms for the establishment of mating pairs, the sizes of mating aggregates are different, and they have different host ranges within gram-negative (F) as well as gram-positive (pCF10 and pXO16) bacteria. Their plasmid sizes are also different; 54, 100 and 200 kb, respectively. Remarkably, however, those conjugation systems have very important characteristics in common: they are able to sustain conjugative transfer in liquid medium and transfer efficiencies close to 100% are often reached in a very short time. Thus, the conjugative process permits the protection of plasmid DNA against environmental nucleases, and the very efficient delivery of plasmid DNA into a recipient cell. Conjugation functions are naturally plasmid encoded. Numerous conjugative plasmids (and transposons) are known, which can transfer associated genes within one species (narrow host range) or between many species (broad host range). Transmissible plasmids have been reported in numerous Gram-positive genera, including but not

limited to pathogenic strains of *Streptococcus, Staphylococcus, Bacillus, Clostridium* and *Nocardia*. The early stages of conjugation generally differ in Gram-negative and Gram-positive bacteria. The role of some of the transfer genes in conjugative plasmids from Gram-negative bacteria are to provide pilus-mediated cell-to-cell contact, formation of a conjugation pore and related morphological functions. The pili do not appear to be involved in initiating conjugation in Gram-positive bacteria.

SUMMARY OF THE INVENTION

The invention provides:

A method (eg, a non-medical or a medical) for enhancing the growth or weight of a subject, wherein the subject comprises bacterial target cells, the method comprising administering to the subject a first episomal DNA encoding an antibacterial agent that is toxic to the target cells, wherein first DNA is transferred into target cells and the agent is expressed, thereby killing target cells in the subject or reducing the growth or proliferation of target cells and enhancing growth or weight of the subject.

In an embodiment, there is provided:

A method (eg, a non-medical or a medical) for enhancing the growth or weight of a subject, wherein the method comprises the administration of a plurality of carrier cells to the subject, wherein the subject comprises bacterial target cells and each carrier cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the agent, wherein first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the subject or reducing the growth or proliferation of target cells and enhancing growth or weight of the subject.

Advantageously, the FCR is improved (ie, enhanced), ie, the FCR number is lowered, in the subject. When the method of the invention is carried out on a group of subjects (eg, a group of animals, such as livestock animals) the FCR is lowered in an individual in the group or the average FCR is lowered in the group. Lowering may be assessed as a comparison with FCR prior to administration of the carrier cell(s) or compared to an average for animals of the same species, age group and when fed on comparable or the same diet. The skilled addressee will be familiar with standard FCRs, such as for livestock animals, eg, piglets, pigs, sheep, cattle (dairy or meat cattle), fish, shellfish, poultry (eg, chickens (broiler or egg-layer hens), geese, ducks or turkeys).

The invention also provides:

A carrier cell, wherein the cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a bacterial target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the antibacterial agent, hereby killing the target cell, wherein the target cell is a *Salmonella* cell and the carrier cell is an *Enterobacteriaceae* cell.

A composition comprising a plurality of carrier cells for use in a method comprising administration of the cells to a subject to treat an infection by pathogenic bacterial target cells, wherein each carrier cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the agent, wherein first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the subject or reducing the growth or proliferation of target cells, wherein the target cells are *Salmonella* cells and the carrier cells are *Enterobacteriaceae* cells.

A non-medical method of killing zoonotic bacterial target cells in an animal (optionally a livestock animal), the method comprising administering to the animal a plurality of carrier cells, wherein each carrier cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the agent, wherein first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the subject or reducing the growth or proliferation of target cells, wherein the target cells are *Salmonella* cells and optionally the carrier cells are *Enterobacteriaceae* cells.

A DNA (optionally for use in the method of the invention), wherein the DNA is capable of being introduced into a target cell, wherein the DNA encodes a plurality of guide RNAs or crRNAs of a CRISPR/Cas system wherein the guide RNAs or crRNAs are operable with Cas nuclease in the target cell to recognise a plurality of protospacer sequences comprised by the target cell genome; wherein

(a) the protospacer sequences comprise one or more pathogenic island nucleotide sequences of the target cell genome;

(b) the protospacer sequences comprise one or more invasion gene sequences of the target cell genome;

(c) the protospacer sequences comprise one or more secretion system gene sequences of the target cell genome; and/or

(d) the protospacer sequences comprise one or more nucleotide sequences of genes selected from A gene selected from *avrA, sptP, sicP, sipA, sipD, sipC, sipB, sicA, invB, ssaE, sseA, sseB, sscA, sseC, sseD, sseE, sscB, sseF, sseG, mgtC, cigR, pipA, pipB, pipC, sopB* and *pipD* (optionally selected from *invB, sicP, sseE, pipA, pipB, pipC, hilA, marT* and *sopB* of *Salmonella*, and orthologues or homologues thereof.

The also invention provides the following configurations:-

In a First Configuration

A carrier bacterial cell comprising a first episomal DNA, the DNA encoding a nucleic acid sequence of interest (NSI) or encoding an antibacterial agent that is toxic to a target bacterial cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into the target cell for expression therein of the agent, optionally wherein

(a) the carrier cell comprises a second DNA which is different from the first DNA, wherein the second DNA comprises or encodes a first factor required for replication of the first DNA;

(b) the first DNA does not comprise or encode said first factor, wherein the first DNA is non-self-replicative in the absence of the first factor, but is able to replicate in the carrier cell in the presence of the first factor provided by the second DNA;

(c) wherein the cell comprises genes encoding one or more conjugation factors sufficient to carry out conjugative transfer of the first DNA into a target bacterial cell.

In embodiments, the invention usefully recognizes the benefit of using antibacterial agents that act by target sequence recognition in the target cell genome but not in the carrier cell, which frees up the ability for the first DNA to be freely replicated in the carrier cell without toxicity to the carrier cell.

In a Second Configuration

A DNA encoding an NSI or encoding an antibacterial agent that is toxic to a target bacterial cell but is not toxic to a carrier cell that is capable of carrying the DNA for conjugative transfer into the target cell, wherein the first DNA does not comprise or encode a first factor required for replication of the first DNA, and wherein the first DNA is devoid of a component required for conjugative transfer of the first DNA into a target bacterial cell.

In a third Configuration

A method for enhancing growth or weight of a human or animal subject, wherein the method comprises the administration of a plurality of carrier cells according to the invention to a microbiota of the subject, wherein the microbiota comprises target cells and first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells (eg, *Salmonella* cells) in the subject or reducing the growth or proliferation of target cells.

In a Fourth Configuration

A method for enhancing growth or weight of a plant, wherein the method comprises the administration of a plurality of carrier cells according to the invention to a microbiota of the plant, wherein the microbiota comprises target cells and first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells (eg, *Pseudomonas* cells) in the plant or reducing the growth or proliferation of target cells.

In a Fifth Configuration

A method for reducing a biofilm comprised by a subject or comprised on a surface, wherein the biofilm comprises target cells (eg, *Pseudomonas* cells), wherein the method comprises the administration of a plurality of carrier cells according to the invention to the biofilm, wherein first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the biofilm or reducing the growth or proliferation of target cells.

In a sixth Configuration

A method of replicating a first DNA (eg, comprised by conjugative plasmids) to produce a plurality of copies of said DNA, the method comprising culturing a plurality of carrier bacterial cells according to the invention, wherein the first DNA is replicated in the cells; and optionally isolating a plurality of copies of the first DNA from carrier cells.

In a seventh Configuration

A method of killing a plurality of target bacterial cells or reducing the growth or proliferation thereof, the method comprising

(a) obtaining a sample of the carrier cells according to the invention or obtainable by the method of the sixth configuration;

(b) contacting the sample of carrier cells with the plurality of target bacterial cells to allow conjugation between carrier cells and target cells; and

(c) allowing copies of first DNA to be transferred by conjugative transfer from carrier cells to target cells, wherein the antibacterial agent is provided in target cells and target cells are killed or the growth or proliferation of target cells is reduced;

(d) wherein the first DNA is not (or not substantially) replicable in the target cells.

In an eighth Configuration

A pharmaceutical composition, livestock growth promoting composition, zoonosis control agent, biocidal agent for administration to livestock, soil improver, herbicide, plant fertilizer, food or food ingredient sterilizing composition, dental composition, personal hygiene composition or disinfectant composition (eg, for domestic or industrial use) comprising a plurality of carrier cells according to the invention.

In an ninth Configuration

In a first aspect:-

A method of promoting the growth of an animal (eg, a livestock animal, eg, a poultry animal), the method comprising administering to the animal a guided nuclease system or a component thereof, and introducing the system or component into target bacteria comprised by the animal, wherein the guided nuclease is capable of recognising and modifying (eg, cutting) a target nucleotide sequence comprised by the target bacteria, whereby target bacteria are killed or the growth or proliferation of target bacteria are inhibited and the growth of the animal is promoted.

The method is a non-medical method and the presence of target bacteria in the animal is capable of inhibiting the growth of the animal. Thus, the method reduces the burden of such bacteria in the animal and promotes growth.

In a second aspect:-

A method of enhancing feed conversion ratio (FCR) in an animal (eg, a livestock animal, eg, a poultry animal), the method comprising administering to the animal a guided nuclease system or a component thereof, and introducing the system or component into target bacteria comprised by the animal, wherein the guided nuclease is capable of recognising and modifying (eg, cutting) a target nucleotide sequence comprised by the target bacteria, whereby target bacteria are killed or the growth or proliferation of target bacteria are inhibited and the FCR of the animal is increased.

The method is a non-medical method and the presence of target bacteria in the animal is capable of increasing the FCR of the animal. Thus, the method reduces the burden of such bacteria in the animal and enhances FCR (ie, reduces FCR number).

In a third aspect:-

A method of promoting the growth of an animal (eg, a livestock animal, eg, a poultry animal), the method comprising administering to the animal an antibacterial agent that is toxic to *Salmonella* bacteria, wherein *Salmonella* target bacteria comprised by the animal are exposed to the agent and are killed or the growth or proliferation of target bacteria are inhibited and the growth of the animal is promoted.

The method is a non-medical method and the presence of target bacteria in the animal is capable of inhibiting the growth of the animal. Thus, the method reduces the burden of such bacteria in the animal and promotes growth.

In a fourth aspect:-

A method of enhancing feed conversion ratio (FCR) in an animal (eg, a livestock animal, eg, a poultry animal), the method comprising administering to the animal an antibacterial agent that is toxic to *Salmonella* bacteria, wherein *Salmonella* target bacteria comprised by the animal are exposed to the agent and are killed or the growth or proliferation of target bacteria are inhibited and the FCR of the animal is enhanced.

The method is a non-medical method and the presence of target bacteria in the animal is capable of increasing the FCR of the animal. Thus, the method reduces the burden of such bacteria in the animal and enhances FCR (ie, reduces FCR number).

In an tenth Configuration

In a first aspect:-

A method of promoting growth, dry weight, wet weight or crop production of a plant, the plant comprising a microbiota that comprises target bacteria, the method comprising contacting the microbiota with an antibacterial agent that is toxic to the target bacteria, wherein target bacteia are killed or the growth or proliferation of target bacteria is inhibited and the growth, dry weight, wet weight or crop production of the plant is increased.

In a second aspect:-
Use of an antibacterial agent that is toxic to target bacteria in a method wherein target bacteria comprised by a microbiota of a plant are contacted with the agent, whereby target bacteria are killed or the growth or proliferation of target bacteria is inhibited, for promoting growth, dry weight, wet weight or crop production of the plant.

In a third aspect:-
A method of increasing crop yield of a plant, the plant comprising a microbiota that comprises target bacteria, the method comprising contacting the microbiota with an antibacterial agent that is toxic to the target bacteria, wherein target bacteia are killed or the growth or proliferation of target bacteria is inhibited and the growth, dry weight, wet weight or crop production of the plant is increased.

In a fourth aspect:-
Use of an antibacterial agent that is toxic to target bacteria in a method wherein target bacteria comprised by a microbiota of a plant are contacted with the agent, whereby target bacteria are killed or the growth or proliferation of target bacteria is inhibited, for increasing crop yield of the plant.

In a fifth aspect:-
A method of increasing leaf chlorophyll of a plant, the plant comprising a microbiota that comprises target bacteria, the method comprising contacting the microbiota with an antibacterial agent that is toxic to the target bacteria, wherein target bacteia are killed or the growth or proliferation of target bacteria is inhibited and the leaf chlorophyll of the plant is increased.

In a sixth aspect:-
Use of an antibacterial agent that is toxic to target bacteria in a method wherein target bacteria comprised by a microbiota of a plant are contacted with the agent, whereby target bacteria are killed or the growth or proliferation of target bacteria is inhibited, for increasing leaf chlorophyll of the plant.

In a seventh aspect:-
A method of increasing greening of a plant, the plant comprising a microbiota that comprises target bacteria, the method comprising contacting the microbiota with an antibacterial agent that is toxic to the target bacteria, wherein target bacteia are killed or the growth or proliferation of target bacteria is inhibited and greening of the plant is increased.

In a eighth aspect:-

Use of an antibacterial agent that is toxic to target bacteria in a method wherein target bacteria comprised by a microbiota of a plant are contacted with the agent, whereby target bacteria are killed or the growth or proliferation of target bacteria is inhibited, for increasing greening of the plant.

In a ninth aspect:-

A method of decreasing a biofilm (eg, a leaf biofilm) comprised by a plant, the plant comprising a biofilm that comprises target bacteria, the method comprising contacting the microbiota with an antibacterial agent that is toxic to the target bacteria, wherein target bacteia are killed or the growth or proliferation of target bacteria is inhibited and the biofilm of the plant is decreased.

In a tenth aspect:-

Use of an antibacterial agent that is toxic to target bacteria in a method wherein target bacteria comprised by a biofilm (eg, a leaf biofilm) of a plant are contacted with the agent, whereby target bacteria are killed or the growth or proliferation of target bacteria is inhibited, for inhibiting the biofilm of the plant.

Optionally, the target bacteria are *Pseudomonas* bacteria, such as *P syringae* or *P aeruginosa* bacteria or any other Pseudomonas bacteria disclosed herein.

Optionally, the agent is a guided nuclease system or a component thereof, eg, any such system or component disclosed herein for modifying (eg, cutting) a target nucleic acid sequence comprised by target bacteria.

Optionally, the plant is any plant disclosed herein.

Optionally, the chlorophyll is a chlorophyll a and/or chlorophyll b.

Optionally, the agent is comprised by a carrier cell of the present invention and said contacting comprises contacting the cell with the carrier cell.

BRIEF DESCRIPTION OF FIGURES

Figure 1. PCRs performed on recipient colonies. From the left to the right: 1) Plasmid pFSMobC* (control). 2) Plasmid pFSMobC* in S17 (control). 3) Colony from transconjugants plate (Cm30+ Nal25). 4) Colony n°2 from transconjugants plate (Cm30+ Nal25);

Figure 2: Effect of viability of pFSMobSal7 delivered by conjugation on Salmonella enteritidis FS26;

Figure 3. Numbers of *Salmonella* in the caecum at 7 days post infection. N=15 birds per group;

Figure 4. Frequency of detection of *Salmonella* in the caecum at 7 days post infection. N=15 birds per group; and

Figure 5. Post mortem carcass weight of birds at 6 weeks of age.

Figure 6. Numbers (CFU/g) of *Salmonella* in crop digesta 7 days post-challenge; N=9 birds; P=0.03.

DETAILED DESCRIPTION

The invention relates to means for carrying out conjugation between bacteria, and in particular the invention relates to carrier bacteria comprising antimicrobial agents and methods of use. A carrier bacterium is capable of conjugative transfer of DNA encoding the agent to a target cell.

The invention further relates to growth, weight or feed conversion ratio (FCR) promotion in animals. The invention further relates to killing *Salmonella* or inhibiting the growth or proliferation of *Salmonella*.

Thus, there is provided:

A method (eg, a non-medical or a medical) for enhancing the growth or weight of a subject, wherein the subject comprises bacterial target cells, the method comprising administering to the subject a first episomal DNA encoding an antibacterial agent that is toxic to the target cells, wherein first DNA is transferred into target cells and the agent is expressed, thereby killing target cells in the subject or reducing the growth or proliferation of target cells and enhancing growth or weight of the subject.

Advantageously, the FCR is enhanced in the subject. Optionally, the FCR is enhanced (ie, lowered) by at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10%, eg from 2 to 6% or from 2 to 5% (such as 2, 3, 4, 5 or 6%) compared to the FCR of the subject prior to administration of the agent or carrier cells. Optionally, the FCR is enhanced by at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10%, eg from 2-5% (such as 2, 3, 4, or 5%) compared to the FCR of a control subject of the same species and sex that has not received administration of the agent or carrier cells. In an embodiment, the subject is a bird, poultry bird, chicken, turkey, goose or duck (preferably a chicken) and the FCR number is lowered by an amount from 0.03 to 0.07, eg, from 0.04 to 0.06, eg, 0.04, 0.05 or 0.06. Thus, for example, compared to a control bird, the FCR of the control bird is 1.7 (g feed/g bird) and after a subject bird that has been treated using the invention has a FCR of 1.64 to 1.66. The control bird (eg, chicken) of the same species, sex and age as the subject of the invention and fed on the same diet (feed), but does not receive the treatment of the invention. The birds may be from the same flock. In an embodiment,

therefore, a bird (eg, chicken) has a FCR of 1.64 to 1.66, wherein the subject has been treated by the method of the invention.

In an example, the method is carried out on a group of subjects (eg, subjects of the same species, such as a group of livestock animals, eg, a poultry flock or herd of cattle or sheep). In this example, the average FCR of the group is enhanced by the method (ie, the FCR number is lowered) compared to a control group of animals of the same species, subspecies or type, or compared to the average FCR of the group prior to treatment with the method. A control group may be a group of animals of the same species, with the same average age, same proportion of males and females and fed on the same diet as the group of the invention. For example, the group of the invention and control group are both poultry (eg, chicken) flocks, eg, with common ancestry one or two or three generations back. The flock may be a flock of broiler chickens or hen-layer hens. For example, the group of the invention and control group are both beef cattle herds, or dairy herds, eg, with common ancestry one or two or three generations back.

FCR, FE & ECI

In animal husbandry, feed conversion ratio (FCR) is a ratio measuring the efficiency with which the bodies of livestock convert animal feed into the desired output. For dairy cows, for example, the output is milk, whereas in animals raised for meat (such as beef cows, pigs, chickens, and fish) the output is the flesh, that is, the body mass gained by the animal, represented either in the final mass of the animal or the mass of the dressed output. FCR is the mass of the input divided by the output (thus mass of feed per mass of milk or meat). In some sectors, Feed Efficiency (FE), which is the output divided by the input (i.e. the inverse of FCR), is used. These concepts are also closely related to efficiency of conversion of ingested foods (ECI). FCR is widely used in swine and poultry production, while FE is used more commonly with cattle. Being a ratio the FCR is dimensionless, that is, it is not affected by the units of measurement used to determine the FCR. Animals that have a low FCR are considered efficient users of feed.

Preferably, the enhancement produced by the invention is an increase in body mass of the subject. Instead of enhancement of FCR, the invention may be a method for enhancing Feed Efficiency (FE) in the subject, or enhancing the Efficiency of Conversion of Ingested Foods (ECI) in the subject.

FCR may be calculated using feed dry mass, or may be calculated on an as-fed wet mass basis.

In an embodiment, the FCR, FE or ECI number is changed by 0.2, 0.5, 1, 1.5 or 2. For example, the FCR is reduced by 1, 1.5 or 2.

Conversion ratios for livestock

Beef cattle

As of 2013 in the US, an FCR calculated on live weight gain of 4.5–7.5 was in the normal range with an FCR above 6 being typical. Thus, in an example of the invention the or each animal is a beef cow and the FCR number in the animal (or average FCR of the animals) is reduced by the method to below 6 (calculated using live weight of the animal), eg, below 5.5, 5, 4.5, 4 or 3.5 (optionally, said FCR or average FCR is reduced to an FCR from 3.5 to 5.9, eg, from 4 to 5.5.

Dairy cattle

In the US, the price of milk is based on the protein and fat content, so the FCR is often calculated to take that into account. Using an FCR calculated just on the weight of protein and fat in milk obtained from the animal(s), as of 2011 an FCR of 13 was poor, and an FCR of 8 was very good. Thus, in an example of the invention the or each animal is a dairy cow and the FCR number in the animal (or average FCR of the animals) is reduced by the method to below 11, 10, 9 or 8, (and optionally greater than 5 or 6) wherein the FCR is the FCR of milk obtained from the animal(s) that have been treated by the method of the invention, the FCR being calculated using the combined weight of protein and fat in the milk.

Another method for dealing with pricing based on protein and fat, is using energy-corrected milk (ECM), which adds a factor to normalize assuming certain amounts of fat and protein in a final milk product; that formula is (0.327 x milk mass) + (12.95 x fat mass) + (7.2 x protein mass). In the dairy industry, Feed Efficiency (ECM/intake) is often used instead of FCR (intake/ECM); an FE less than 1.3 is considered problematic. Thus, in an example of the invention the or each animal is a dairy cow and the FE number in the animal (or average FE of the animals) is increased by the method to above 1.3, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5 or 3, (and optionally no greater than 4 or 5). Optionally, the FE is increased above 1.3, 1.5, 1.6 and no greater than 1.7.

FE based simply on the weight of milk is also used; an FE between 1.30 and 1.70 is normal. Thus, in an example of the invention the or each animal is a dairy cow and the FE number in the animal (or average FE of the animals) is increased by the method to above 1.3, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5 or 3, (and optionally no greater than 4 or 5). Optionally, the FE is increased above 1.3, 1.5, 1.6 and no greater than 1.7.

Pigs

As of 2011, pigs used commercially in the UK and Europe had an FCR, calculated using weight gain, of about 1 as piglets and ending about 3 at time of slaughter. As of 2012 in Australia and using dressed weight for the output, a FCR calculated using weight of dressed meat of 4.5 was fair, 4.0 was considered "good", and 3.8, "very good". In the US as of 2012, commercial pigs had FCR calculated using weight gain, of 3.46 for while they weighed between 240 and 250 pounds, 3.65 between 250 and 260 pounds, 3.87 between 260 and 270 lbs, and 4.09 between 280 and 270 lbs. Thus, in an example of the invention the or each animal is a piglet and the FCR number in the animal (or average FCR of the animals) is reduced by the method to 1 or less, the FCR being calculated using weight gain of the animal(s). Thus, in an example of the invention the or each animal is a pig up to 3 months old and the FCR number in the animal (or average FCR of the animals) is reduced by the method to 1 or less, the FCR being calculated using weight gain of the animal(s). Thus, in an example of the invention the or each animal is a pig greater than 3 months old, but up to 6 or 7 months' old, and the FCR number in the animal (or average FCR of the animals) is reduced by the method to 3 or less (eg, from 1 to 3), the FCR being calculated using weight gain of the animal(s). A piglet may be from 1.5 to 3 months' of age; a pig for slaughter may be greater than 3, but up to 6 months' of age. Thus, in an example of the invention the or each animal is a pig whose weight is between 240 and 250 pounds and the FCR number in the animal (or average FCR of the animals) is reduced by the method to 3.5 or less (eg, 3.4 or less, but optionally no less than 3 or 2.5), the FCR being calculated using weight gain of the animal(s). Thus, in an example of the invention the or each animal is a pig whose weight is between 250 and 260 pounds and the FCR number in the animal (or average FCR of the animals) is reduced by the method to 3.7 or less (eg, 3.65 or less, but optionally no less than 3 or 3.5), the FCR being calculated using weight gain of the animal(s). Thus, in an example of the invention the or each animal is a pig whose weight is between 260 and 270 pounds and the FCR number in the animal (or average FCR of the animals) is reduced by the method to 3.9 or less (eg, 3.8 or less, but optionally no less than 3.7 or 3.5), the FCR being calculated using weight gain of the animal(s). Thus, in an example of the invention the or each animal is a pig whose weight is between 280 and 270 pounds and the FCR number in the animal (or average FCR of the animals) is reduced by the method to 4.1 or less (eg, 4 or less, but optionally no less than 3.8 or 3.9), the FCR being calculated using weight gain of the animal(s).

Sheep

Some data for sheep illustrate variations in FCR. A FCR (kg feed dry matter intake per kg live mass gain) for lambs is often in the range of about 4 to 6. Thus, in an example of the invention the or each

animal is a sheep and the FCR number in the animal (or average FCR of the animals) is reduced by the method to 6 or less (eg, from 4 to 6), the FCR being calculated using kg feed dry matter intake per kg live mass gain of the animal(s).

Poultry

As of 2011 in the US, broiler chickens typically have an FCR of 1.6 based on body weight gain, and mature in 39 days. At around the same time the FCR based on weight gain for broilers in Brazil was 1.8. Thus, in an example of the invention the or each animal is a poultry bird (eg, a broiler chicken) and the FCR number in the animal (or average FCR of the animals) is reduced by the method to 1.9, 1.8, 1.7, 1.6 or less (eg, from 1.9 to 1.5; or 1.8 to 1.6), the FCR being calculated based on body weight gain. Optionally, the FCR is calculated when each animal is 35-40, eg, 39 days old.

For hens used in egg production in the US, as of 2011 the FCR was about 2, with each hen laying about 330 eggs per year. Thus, in an example of the invention the or each animal is a poultry bird (eg, an egg-laying hen, eg, a chicken) and the FCR number in the animal (or average FCR of the animals) is reduced by the method to 2 or less (eg, from 2 to 1.5), the FCR being calculated based on body weight gain. Thus, in an example of the invention the or each animal is a poultry bird (eg, an egg-laying hen, eg, a chicken) and the number of eggs layed by the animal (or average number of eggs layed by the animals) is more than 330 eggs per year (or pro rated for a different period), eg, more than 340, 350 or 400 eggs per year (or pro rated period).

Carnivorous fish

The FIFO ratio (or Fish In – Fish Out ratio) is a conversion ratio applied to aquaculture, where the first number is the mass of harvested fish used to feed farmed fish, and the second number is the mass of the resulting farmed fish. FIFO is a way of expressing the contribution from harvested wild fish used in aquafeed compared with the amount of edible farmed fish, as a ratio. Fishmeal and fish oil inclusion rates in aquafeeds have shown a continual decline over time as aquaculture grows and more feed is produced, but with a finite annual supply of fishmeal and fish oil. Calculations have shown that the overall fed aquaculture FIFO declined from 0.63 in 2000 to 0.33 in 2010, and 0.22 in 2015. In 2015, approximately 4.55 kg of farmed fish was produced for every 1 kg of wild fish harvested and used in feed. The fish used in fishmeal and fish oil production are not used for human consumption, but with their use as fishmeal and fish oil in aquafeed they contribute to global food production. Thus, in an example the method of the invention enhances (ie, increases) the FIFO ratio when the animal if a fish (eg, a salmon, tilapia or catfish). For example, the FIFO is increased to 0.3, 0.4, 0.5, 0.6, 0.7 or 0.8, 1, 1.5 or 2 or more. For example, the fish is a salmon or catfish and the FIFO is raised above 1,

1.5 or 2 (and optionally no more than 2 or 2.5). For example, the fish is a talapia and the FIFO is raised above 1.5 or 2 (and optionally no more than 2 or 2.5).

Enhancement of growth or weight may be an enhancement (increase) in milk production or yield, eg, an average increase for a group of the invention compared with a control group. A control group may be a group of animals of the same species, with the same average age, same proportion of males and females and fed on the same diet as the group of the invention. For example, here the subjects may be dairy cattle.

Enhancement of growth or weight may be an enhancement (increase) in egg production or yield, eg, an average increase for a group of the invention compared with a control group. A control group may be a group of animals of the same species, with the same average age, same proportion of males and females and fed on the same diet as the group of the invention. For example, here the subjects may be hen-layer chickens.

Enhancement of growth or weight may be an enhancement (increase) in meat production or yield, eg, an average increase for a group of the invention compared with a control group. A control group may be a group of animals of the same species, with the same average age, same proportion of males and females and fed on the same diet as the group of the invention. For example, here the subjects may be dairy cattle, poultry (eg, chickens), fish, shellfish, sheep or pigs.

Enhancement of growth or weight may be an enhancement (increase) in fat production or yield, eg, an average increase for a group of the invention compared with a control group. A control group may be a group of animals of the same species, with the same average age, same proportion of males and females and fed on the same diet as the group of the invention. For example, here the subjects may be dairy cattle, poultry (eg, chickens), fish (eg, salmon, talapia or catfish), shellfish, sheep or pigs.

Enhancement of growth or weight may be an enhancement (increase) in fur or hide production or yield, eg, an average increase for a group of the invention compared with a control group. A control group may be a group of animals of the same species, with the same average age, same proportion of males and females and fed on the same diet as the group of the invention. For example, here the subjects may be cows.

In an example, the subject is a shellfish. The shellfish may be selected from Shrimp, crayfish, crab, lobster, clam, scallop, oyster, prawn and mussel.

The subject may be any subject disclosed herein. The subject may be an animal, such as a livestock animal, eg, a bird (such as a poultry bird; or a chicken or a turkey) or swine. Alternatively, the subject may be a human, eg, a human suffering from an eating disorder (such as anorexia) or who is underweight, eg, wherein the human has a body mass index (BMI) less than 18.5, 18, 17, 16 or 15. For example, the human has mild anorexia (ie, the human has a BMI<17.5), moderate anorexia (ie, the human has a BMI of from16 to 16.99), severe anorexia (ie, the human has a BMI of from15 to 15.99) or extreme anorexia (ie, the human has a BMI <15).

In an alternative, the subject is a plant, eg, and the target bacteria are plant pathogen bacteria. In an example, the target baceteria are *Pseudomonas*, eg, *P syringae* or *P aeruginosa*.

In an alternative, the target cells are archaeal cells. For example the target cells are methanobacterium cells.

For example the target cells are methanogen cells. For example, the target cells comprise one or more species of cell selected from:

- *Methanobacterium bryantii*
- *Methanobacterium formicum*
- *Methanobrevibacter arboriphilicus*
- *Methanobrevibacter gottschalkii*
- *Methanobrevibacter ruminantium*
- *Methanobrevibacter smithii*
- *Methanococcus chunghsingensis*
- *Methanococcus burtonii*
- *Methanococcus aeolicus*
- *Methanococcus deltae*
- *Methanococcus jannaschii*
- *Methanococcus maripaludis*
- *Methanococcus vannielii*
- *Methanocorpusculum labreanum*
- *Methanoculleus bourgensis (Methanogenium olentangyi & Methanogenium bourgense)*
- *Methanoculleus marisnigri*
- *Methanoflorens stordalenmirensis[34]*
- *Methanofollis liminatans*

- *Methanogenium cariaci*
- *Methanogenium frigidum*
- *Methanogenium organophilum*
- *Methanogenium wolfei*
- *Methanomicrobium mobile*
- *Methanopyrus kandleri*
- *Methanoregula boonei*
- *Methanosaeta concilii*
- *Methanosaeta thermophila*
- *Methanosarcina acetivorans*
- *Methanosarcina barkeri*
- *Methanosarcina mazei*
- *Methanosphaera stadtmanae*
- *Methanospirillium hungatei*
- *Methanothermobacter defluvii (Methanobacterium defluvii)*
- *Methanothermobacter thermautotrophicus (Methanobacterium thermoautotrophicum)*
- *Methanothermobacter thermoflexus (Methanobacterium thermoflexum)*
- *Methanothermobacter wolfei (Methanobacterium wolfei)*
- *Methanothrix sochngenii*

Optionally, the target cells are not pathogenic to the subject, for example when the method is a non-medical method. In an example, the method is a cosmetic method.

For example the target cells are methane-producing cells, and optionally the subject is a livestock animal, preferably a ruminant, or a cow (eg, a beef or dairy cattle). By reducing methane-producing cells in such animal, the invention may in one embodiment enhance the weight of the animal (eg, enhance the yield of meat from the animal) and/or enhance the yield of milk or another product of the animal, such as fur or fat.

In an example, the target cells are selected from *E coli, Salmonella* and *Campylobacter* cells. In an example, the target cells are *E coli, Salmonella* or *Campylobacter* cells. In an example, each animal is a chicken (eg, a broiler or hen-layer) and the target cells are *Salmonella* or *Campylobacter* cells. In an example, each animal is a cow (eg, a beef or dairy cow) and the target cells are mehanogen cells.

In an example, the target cells are selected from Mycoplasma (eg, *Mycoplasma mycoides* (eg, *Mycoplasma mycoides* subsp. *Mycoides*), *Mycoplasma leachii* or *Mycoplasma bovis*), *Brucella abortus, Listeria monocytogenes, Clostridium* (eg, *Clostridium chauvoei* or *Clostridium septicum*), *Leptospira* (eg, L. *canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. hardjo* or *L. Pomona*), *Mannheimia haemolytica, Trueperella pyogenes, Mycobacterium bovis, Campylobacter* spp. (eg, *Campylobacter jejuni* or *Campylobacter coli*), *Bacillus anthracis*, *E. coli* (eg, *E. coli* O157:H7) or *Pasteurella multocida* (eg, *Pasteurella multocida* B:2, E:2, A:1 or A:3). In the example, optionally the subject or animal is a livestock animal, such as a cow, sheep, goat or chicken (preferably a cow).

Optionally, eg, wherein the subject is an animal (eg, a livestock animal or a wild animal), the target cells are zoonotic bacterial cells, such as cells of a species selected from Bacillus anthracis, Mycobacterium bovis (eg, wherein the animal is a cow), Campylobacter spp (eg, wherein the animal is a poultry animal), Mycobacterium marinum (eg, wherein the animal is a fish), Shiga toxin-producing E. Coli (eg, wherein the animal is a ruminant), Listeria spp (eg, wherein the animal is a cow or sheep), Chlamydia abortus (eg, wherein the animal is a sheep), Coxiella burnetii (eg, wherein the animal is a cow, sheep or goat), Salmonella spp (eg, wherein the animal is a poultry animal), Streptococcus suis (eg, wherein the animal is a pig) and Corynebacterium (eg, C ulcerans) (eg, wherein the animal is a cow).

In an example, a plurality of carrier cells as described herein (eg, carrier cells of any configuration, aspect, example or embodiment described herein) is administered to the subject, wherein the carrier cells comprise the DNA encoding the agent.

In an example, each animal is a chicken (eg, a broiler or hen-layer) and the target cells are *Salmonella* or *Campylobacter* cells. In an example, each animal is a cow (eg, a beef or dairy cow) and the target cells are mehanogen cells.

In an embodiment, therefore, there is provided:

A method (eg, a non-medical or a medical) for enhancing the growth or weight of a subject, wherein the method comprises the administration of a plurality of carrier cells to the subject, wherein the subject comprises bacterial target cells and each carrier cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the agent, wherein first DNA is transferred from carrier cells into target cells for

expression therein to produce the antibacterial agent, thereby killing target cells in the subject or reducing the growth or proliferation of target cells and enhancing growth or weight of the subject.

Optionally, the target cells are *Salmonella* cells. In an example, the target cells comprise *S enterica* and/or *S typhimurium* cells; optionally wherein the *S enterica* is *S enterica* subspecies *enterica*. Optionally, the method kills a plurality of different *S enterica* subspecies *enterica* serovars; optionally wherein each serovar is selected from the group consisting of Typhimurium, Enteritidis, Virchow, Montevideo, Heidelberg, Hadar, Binza, Bredeney, Infantis, Kentucky, Seftenberg, Mbandaka, Anatum, Agona and Dublin. Optionally, the method kills *S enterica* subspecies *enterica* serovars Typhimurium, Infantis and Enteritidis. Optionally, the method kills *S enterica* subspecies *enterica* serovars Typhimurium and Enteritidis. Optionally, the method kills *S enterica* subspecies *enterica* serovars Typhimurium and Infantis. Optionally, the method kills *S enterica* subspecies *enterica* serovars Enteritidis and Infantis. The most prevalent serovars in chicken are *Salmonella Enteritidis*, *Salmonella Infantis* and *Salmonella Typhimurium*. In general, similar serovars of Salmonella are found in infected humans and chicken (*S. Enteritidis* and *S. Typhimurium*). By killing Salmonella in livestock animals, the invention is useful for reducing the pool of zoonotic bacteria that are available for transmission to humans (such as by eating the livestock or products made thereofrom, such as meat or dairy products for human consumption).

Advantageously, the carrrier cells are *Enterobacteriaceae* cells, optionally *E coli* cells. As exemplified by the Examples below, the inventors have surprisingly found that (i) antibacterial agents can be efficiently transferred from such cells to Salmonella target cells both *in vivo* and *in vitro*; and (ii) 100% killing of Salmonella was possible when the agent is transferred by conjugation from *Enterobacteriacae cells* to *Salmonella* cells. Furthermore, surprisingly DNA encoding a guided nuclease antibacterial was capable of killing 18 *Salmonella* spp. Serotypes, including the clinically- and zoonotically-important Typhimurium, Infantis and Enteritidis. Optionally, the method kills *S enterica* subspecies *enterica* serovars Typhimurium and Enteritidis serovars. Optionally, the *Enterobacteriaciae* cells are cells of an *Enterobacteriaciae* species shown in Table 5.

Importantly, reduction of target cells was observed in the GI tract of the subject. Thus, optionally the method reduces target cells in the gastrointestinal tract of the animal; optionally the method reduces target cells in the jejunum, ileum, colon, liver, spleen or caecum of the animal; optionally wherein the animal is a bird and the method reduces target cells in the caecum of the bird. This may be important to reduce spread of zoonotic or other deterimental target strains in the faeces of the subjects, such as livestock animals. Thus, in an example the method is carried out on a group of subjects (eg, a herd or

flock, such as a herd of swine or a flock of birds), wherein spread of cells of the target species is reduced in the group. The inventors have also demonstrated this in a flock of poultry where *Salmonella* were killed using the invention, wherein a guided nuclease was used to cut a plurality of protospacer sequences in target cells, thereby killing the cells and reducing spread thereof in the flock.

Thus, in an example the method is carried out on a group (optionally a flock or herd) of animals, wherein some or all of the animals comprise target cells, wherein spread of cells of the target species is reduced in the group; or wherein spread is reduced from the group to a second group of animals.

Optionally, the first DNA is comprised by a plasmid, for example wherein the plasmid comprises a RP4 origin of transfer (oriT). The plasmid may be any type of plasmid disclosed herein.

The agent may be any antibacterial agent disclosed herein, preferably a guided nuclease that is programmed to cut one or more target sequences in target cells. A suitable nuclease may be a TALEN, meganuclease, zinc finger nuclease or Cas nuclease. For example, the agent comprises one or more components (eg, a Cas nuclease and/or a guide RNA or a crRNA) of a CRISPR/Cas system that is operable in a target cell to cut a protospacer sequence comprised by the target cell, optionally wherein the target cells comprise first and second strains of a bacterial species and each strain comprises the protospacer sequence, wherein cells of the strains are killed. For example, the system is operable to cut at least 3 different protospacer sequences comprised by the cell genome. Optionally, each or some of said protospacer sequences is comprised by a pathogenicity island that is comprised by the cell. As shown in the Examples, this is highly effective for target cell killing. Optionally, the agent is operable to cut a plurality of different protospacer sequences comprised by the target cell genome. Optionally, the agent comprises one or more components of a CRISPR/Cas system that is operable in a target cell to cut at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 different protospacer sequences comprised by the target cell genome (eg, comprised by the target cell chromosome).

In an embodiment, the agent

(a) comprises a guided nuclease that is capable of recognising and modifying a target nucleic acid sequence, wherein the target sequence is comprised by an endogenous chromosome or episome of the target cells but is not comprised by the carrier cells, wherein the nuclease modifies the chromosome or episome to kill the target cells or inhibit the growth or proliferation of the target cells; and/or

(b) encodes a guide RNA or crRNA of a CRISPR/Cas system that operates with a Cas nuclease in the target cells to cut a protospacer sequence comprised by the target cells.

There is also provided:

A carrier cell (optionally for use in a method of the invention), wherein the cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a bacterial target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the antibacterial agent, hereby killing the target cell, wherein the target cell is a *Salmonella* cell and the carrier cell is an *Enterobacteriaceae* cell.

There is also provided:

A composition comprising a plurality of carrier cells for use in a method comprising administration of the cells to a subject to treat an infection by pathogenic bacterial target cells, wherein each carrier cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the agent, wherein first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the subject or reducing the growth or proliferation of target cells, wherein the target cells are *Salmonella* cells and the carrier cells are *Enterobacteriaceae* cells. In an alternative, the method treats or reduces a symptom of an infection by pathogenic target cells.

Any administration of cells to a subject herein may be by oral administration. Any administration of cells to a subject herein may preferably be by administration to the GI tract. Any administration of cells to a subject herein may be by systemic, intranasal or inhaled administration.

There is also provided:

A non-medical method of killing zoonotic bacterial target cells in an animal, the method comprising administering to the animal a plurality of carrier cells, wherein each carrier cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the agent, wherein first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the subject or reducing the growth or proliferation of target cells, wherein the target cells are *Salmonella* cells and optionally the carrier cells are *Enterobacteriaceae* cells.

The animal may be any animal disclosed herein, eg, a livestock animal, domesticated animal or wild animal (eg, a bat or bird)).

Optionally, any method herein reduces *Salmonella* in the gastrointestinal tract of the subject. Optionally, the target cells comprise different *Salmonella* spp. types that are killed.

In any aspect, configuration, example, concept or embodiment, the carrier cell, target cell(s) or DNA is respectively a carrier cell, target cell(s) or DNA as defined in any other aspect, configuration, example, concept or embodiment.

The invention also provides:

A DNA, wherein the DNA is capable of being introduced into a target cell, wherein the DNA encodes a plurality of guide RNAs or crRNAs of a CRISPR/Cas system wherein the guide RNAs or crRNAs are operable with Cas nuclease in the target cell to recognise a plurality of protospacer sequences comprised by the target cell genome, optionally wherein the target cell is a *Salmonella* cell or a cell of a species disclosed in Table 5; and

(a) the protospacer sequences comprise one or more pathogenic island nucleotide sequences of the target cell genome;

(b) the protospacer sequences comprise one or more invasion gene sequences of the target cell genome;

(c) the protospacer sequences comprise one or more secretion system gene sequences of the target cell genome; and/or

(d) the protospacer sequences comprise one or more nucleotide sequences of genes selected from *avrA, sptP, sicP, sipA, sipD, sipC, sipB, sicA, invB, ssaE, sseA, sseB, sscA, sseC, sseD, sseE, sscB, sseF, sseG, mgtC, cigR, pipA, pipB, pipC, sopB* and *pipD* (optionally selected from *invB, sicP, sseE, pipA, pipB, pipC, hilA, marT* and *sopB)* of *Salmonella* (eg, *S enterica*) or orthologues or homologues of said genes.

Homologue: A gene, nucleotide or protein sequence related to a second gene, nucleotide or protein sequence by descent from a common ancestral DNA or protein sequence. The term, homologue, may apply to the relationship between genes separated by the event of or to the relationship between genes separated by the event of genetic duplication.

Orthologue: Orthologues are genes, nucleotide or protein sequences in different species that evolved from a common ancestral gene, nucleotide or protein sequence by speciation. Normally, orthologues retain the same function in the course of evolution.

By "orthologues or homologues of said genes" it is meant that a protospacer may be a sequence comprised by a gene of the target cell genome, wherein the gene is an orthologue or homologue of a *Salmonella* gene selected from *avrA, sptP, sicP, sipA, sipD, sipC, sipB, sicA, invB, ssaE, sseA, sseB, sscA, sseC, sseD, sseE, sscB, sseF, sseG, mgtC, cigR, pipA, pipB, pipC, sopB* and *pipD* (optionally selected from *invB, sicP, sseE, pipA, pipB, pipC, hilA, marT* and *sopB).*

Optionally the DNA is the first DNA for use in the method of the invention. Preferably, the target cell is a *Salmonella* cell, eg, a *S enterica* cell, such as a *Salmonella enterica* subsp. *enterica* serovar Enteritidis cell.

Optionally any Salmonella herein is *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2.

Optionally, the target cell is a cell of a species disclosed in Table 5 other than a Salmonella species and the sequences of (d) are sequences of genes selected from orthologues or homologues of *avrA, sptP, sicP, sipA, sipD, sipC, sipB, sicA, invB, ssaE, sseA, sseB, sscA, sseC, sseD, sseE, sscB, sseF, sseG, mgtC, cigR, pipA, pipB, pipC, sopB* and *pipD* (optionally selected from *invB, sicP, sseE, pipA, pipB, pipC, hilA, marT* and *sopB).* of *Salmonella* (eg, *S enterica*).

The DNA may comprise one or more CRISPR spacers, wherein each spacer consists of a nucleotide sequence of a secretion system gene (optionally a type III protein secretion system or a secretion system of SPI-1 or SPI-2 of *Salmonella*); or a T3SS locus gene; or with up to 10, 9, 8, 7, 6, 5, 4, 3 or 2 nucleotide differences therefrom.

Optionally, the DNA (eg, DNA of the carrier cell) encodes a plurality of guide RNAs or crRNAs of a CRISPR/Cas system wherein the guide RNAs or crRNAs are operable with Cas nuclease in the target cell to recognise a plurality of protospacer sequences comprised by the target cell genome, wherein the target cell is a *Salmonella* cell and the protospacer sequences comprise one or more nucleotide sequences of genes selected from *invB, sicP* and *sseE.* For example, the protospacer sequences comprise nucleotide sequences of genes *invB, sicP* and *sseE.* In an example, the DNA also encodes a Cas, eg, a Cas9, Cas3, Cpf1, Cas12, Cas13, CasX or CasY. In an embodiment, the Cas is a Type I, II, III, IV, V or VI Cas, preferably a Type I or II Cas. In an example, the DNA also encodes a Cas3 and

cognate Cascade proteins (eg, CasA, B, C, D and E). Optionally, the Cas (and Cascade of present) are *E coli* Cas (and Cascade).

Optionally, the gene of the target cell encodes a chaperone or secreted effector protein, eg, such a protein encoded by a pathogenicity island or type III protein secretion system (optionally a T3SS locus, such as a secretion system of SPI-1 and SPI-2 of *Salmonella*).

The DNA may comprise one or more CRISPR spacers, wherein each spacer consists of 20-40, 25-35, or 30-35 consecutive nucleotides of a gene comprised by the genome of the target cell; eg,

(a) a gene selected from *avrA, sptP, sicP, sipA, sipD, sipC, sipB, sicA, invB, ssaE, sseA, sseB, sscA, sseC, sseD, sseE, sscB, sseF, sseG, mgtC, cigR, pipA, pipB, pipC, sopB* and *pipD* of *Salmonella* or a homologue or orthologue thereof;

(b) a gene comprised by a pathogenicity island that is comprised by the target cell genome;

(c) a secretion system (eg, a type III protein secretion system) gene comprised by the target cell genome

Optionally, the DNA is comprised by a plasmid which comprises an origin of transfer (oriT) and an origin of replication (oriV) that is operable for replication of the DNA in a bacterial host cell. Optionally, the first DNA is comprised by a plasmid, wherein the plasmid comprises a RP4 origin of transfer (oriT) and/or a p15A origin of replication.

Optionally, the DNA comprises SEQ ID NO: 15, optionally wherein the DNA is comprised by a plasmid in a carrier bacterial cell for conjugation to a *Salmonella* target cell. In an example, the DNA of the invention is comprised by a conjugative plasmid or phagemid.

In an example, the DNA comprises CRISPR repeat and spacer sequences, wherein

(e) the repeat sequences each comprise SEQ ID NO: 16; and/or

(f) the spacer sequences comprise one, two or three sequences selected from SEQ ID NOs: 17-19 and complement sequences thereof;

optionally wherein the DNA is comprised by a plasmid in a carrier bacterial cell for conjugation to a *Salmonella* target cell.

Optionally, the DNA comprises (optionally in 5' to 3' order) SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19. Optionally, the DNA comprises one or more (eg, at least 3) spacer sequences shown in Table 6.

In an example, the DNA encodes a Cas3 and optionally one or more Cascade proteins (eg, one or more of CasA, B, C, D and E). In an embodiment, the first DNA encodes a Cas3 and CasA, B, C, D and E. In an embodiment, the first DNA encodes an *E coli* Cas3 and CasA, B, C, D and E. Optionally, the guided nuclease (eg, Cas3) is a Type I-A, -B, -C, -D, -E, -F or -U Cas.

In an example, the agent in any configuration, example, option or embodiment herein, the agent comprises one or more components of a CRISPR/Cas system that is operable in the target cell to cut a protospacer sequence comprised by the target cell.

In an example, the system is operable to cut at least 3 different protospacer sequences comprised by the target cell genome. In an embodiment, each or some of said protospacer sequences is comprised by a pathogenicity island that is comprised by the target cell.

In an example, the agent in any configuration, example, option or embodiment herein

(a) comprises a guided nuclease that is capable of recognising and modifying a target nucleic acid sequence, wherein the target sequence is comprised by an endogenous chromosome or episome of the target cells but is not comprised by the carrier cells, wherein the nuclease modifies the chromosome or episome to kill the target cells or inhibit the growth or proliferation of the target cells; and/or

(b) encodes a guide RNA or crRNA of a CRISPR/Cas system that operates with a Cas nuclease in the target cell to cut a protospacer sequence comprised by the target cell.

Optionally, the DNA comprises a constitutive promoter for expression of the guide RNAs or crRNAs. Optionally, the DNA comprises a constitutive promoter for expression of a Cas nuclease that is operable in a target cell with the guide RNAs or crRNAs to modify (eg, cut) protospacer sequences of the target cell genome.

Optionally, the Cas, Cascade proteins, gRNAs and crRNAs are E. coli K12 (MG1655) Cas, Cascade proteins, gRNAs and crRNAs respectively. Optionally, the DNA is devoid of nucleotide sequences encoding Cas1 and Cas2 proteins.

The invention also relates to carrier bacteria encoding desired protein or RNA (eg, encoding an antimicrobial agent) and methods of use. In embodiments, the agent can be transferred into target cells by conjugation between carrier cells (to which the agent is not toxic) and the target cells, whereby the agent is toxic to the target cells and kills the target cells. In other embodiments, the growth or proliferation of target cells is reduced (eg, by at least 40, 50, 60, 70, 80, or 90% compared to growth in the absence of the agent). Each carrier cell comprises episomal DNA encoding an antibacterial agent that is toxic to a target bacterial cell but is not toxic (or is less toxic) to the carrier cell. The invention finds application, for example, in controlling or killing target bacteria that are pathogenic to humans, animals or plants. The invention finds application, for example, in controlling or killing zoonotic target bacteria comprised by an animal (eg, a livestock animal). For example, the carrier cells may be comprised by a medicament for treating or preventing a disease or condition in a human or animal; a growth promoting agent for administration to animals for promoting growth theref; killing zoonositic bacteria in the animals; for administration to livestock as a pesticide; a pesticide to be applied to plants; or a plant fertilizer.

An advantage of the invention is that the carrier cells may be used as producer cells in which DNA encoding the antibacterial agent can be replicated. Another advantage of an example of the invention is that further replication of the DNA can be avoided in target cells that do not comprise one or more factors that are required for such replication. Thus, a scheme can be envisaged wherein the factor(s) are present in the carrier cells (and DNA encoding the agent is replicated to provide many copies for subsequent conjugative transfer into target cells) and in the target cells the DNA is not further replicated, which is useful for containining the action of the antibacterial agent, such as in environments or in a human or animal body or in (or on) a plant.a Containment may be required to avoid undesired killing of non-target cells or to provide control generally over dosing and/or the administration of the killing activity regime.

The first DNA is replicable in the carrier cell (but not in the target cell), in which case it is important that the agent is not toxic to the carrier cell, whilst it is toxic to the target cell. In certain embodiments, the invention uses sequence-specific killing of the target cell to achieve this selectivity. To this end, in an example the first DNA encodes a guided nuclease that is operable in the target cell to recognize and cut a target sequence of a target cell chromosome, thereby killing the cell or wherein the growth or proliferation of the cell is reduced. This is advantageous over the use of other types of toxic agent, which are less discriminate in their action, being able to kill several species or strain (eg, potentially also being toxic to the carrier cell to some degree). By using a guided nuclease (eg, a TALEN or Cas nuclease), these can be programmed to recognize a target sequence that is present in

the target cell genome (eg, comprised by a chromosome or episome of the target cell), but is absent in the genome of the carrier cell.

Thus, in this case replication of the first DNA can freely happen in the carrier cell without risk of killing the cell or reducing its growth or proliferation due to the encoded agent and replication of sequences encoding the agent. Thus, in an example, where the first DNA encodes a guided nuclease, the guided nuclease is capable of recognizing and cutting a target nucleic acid sequence comprised by the genome (eg, chromosome) of the target cell, wherein the target cell is absent in the carrier cell.

A particularly useful example is where the first DNA encodes a Cas nuclease (eg, a Cas9 or Cas3) that is operable with a guide RNA or crRNA in the target cell, wherein the RNA is operable to guide the Cas to the target sequence, wherein the Cas modifies (eg, cuts) the target sequence and the target cell is killed or target cell growth or proliferation is inhibited. In one embodiment, the first DNA encodes the Cas and the guide RNA or crRNA. In another embodiment, the first DNA encodes the guide RNA or crRNA, but does not encode a cognate Cas. In this embodiment, the RNA is operable in the target cell with an endogenous Cas encoded by the target cell genome, wherein the RNA is operable to guide the Cas to the target sequence, wherein the Cas modifies (eg, cuts) the target sequence and the target cell is killed or target cell growth or proliferation is inhibited. In this se sense, the agent may comprise a component of a CRISPR/Cas system (eg, a Cas nuclease, Cascade Cas, crRNA, guide RNA or tracrRNA). Thus, the invention usefully recognizes the benefit of using antibacterial agents that act by target recognition in the target cell but not in the carrier cell, which opens up the ability for the first DNA to freely replicated in the carrier cell without significant toxicity to the carrier cell.

Example Plasmids

A method of delivery of any agent, such as a CRISPR-Cas system (or a component thereof) can be by bacterial conjugation, a natural process whereby a donor bacterium transfers DNA from itself to a recipient bacterium. Donor bacteria elaborate a surface structure, the pilus which can be considered to be like a syringe or drinking straw through which the DNA is delivered. The donor pilus binds to the surface of a receptive recipient and this event triggers the process of DNA transfer. Plasmids are suitable for this conjugative process, where the plasmid comprises DNA enoding the agent of the invention.

DNA transfer by conjugation may only take place with a 'susceptible recipient' but does not generally occur with a recipient carrying a similar type of plasmid. Because conjugation is via pilus bridge, it is possible for that bridge to attach itself not to a recipient but to the donor bacterium. This could result

in a futile cycle of transfer of the plasmid DNA to itself. Plasmids thus naturally encode incompatibility factors. One is a surface arrayed protein that prevents the pilus binding to bacterium displaying that surface protein such as itself or any other bacterium carrying the same plasmid. Additionally, plasmids naturally encode another incompatibility system that closely regulates the copy number of the plasmid inside a bacterium. Thus, should a conjugation event manage to evade surface exclusion and start to transfer DNA by conjugation, the recipient will prevent that plasmid establishing as it already maintains the current copy number and will not accept and maintain a further unwanted additional copy.

In an example of the invention, the DNA encoding the agent is comprised by a plasmid. In an embodiment, the plasmid is a member of a plasmid incompatibility group, wherein the target cell does not comprise a plasmid of said group. Optionally, the plasmid of the invention is a member of the incompatibility group P (ie, the plasmid is an *incP* plasmid). *Salmonella* very rarely carry *incP* plasmids, so this *incP* plasmid is useful where the target cell is a *Salmonella* cell. For example within the *Enterobacteriaceae* the following is a non-exclusive list of potential plasmids that could use for delivery: *IncFI, IncFII, IncFIII, IncFIV, IncFV, IncM, Inc9, InclO, Incl, IncA, IncB, IncC, IncH, IncIa, InclIc, IncI2, IncIy, IncJ, IncL, IncN, Inc2e, IncO, IncP, IncS, IncT* and/or *IncW*. Thus, optionally, the target cell is an *Enterobacteriaceae* cell and the DNA of the invention is comprised by a plasmid, wherein the plasmid is selected from an *IncFI, IncFII, IncFIII, IncFIV, IncFV, IncM, Inc9, InclO, Incl, IncA, IncB, IncC, IncH, IncIa, InclIc, IncI2, IncIy, IncJ, IncL, IncN, Inc2e, IncO, IncP, IncS, IncT* and *IncW* plasmid.

In an example, the carrier cell of the invention comprises two or more plasmids, each plasmid comprising a DNA of the invention that encodes an antibacterial agent, wherein a first of said plasmids is a member of a first incompatibility group, wherein the target cell does not comprise a plasmid of said first group, and wherein a second of said plasmids is a member of a second incompatibility group, wherein the target cell does not comprise a plasmid of said second group. For example, a carrier cell may comprise an *incP* plasmid encoding an anti-target cell CRISPR-Cas system or a component thereof (eg, encoding a first crRNA or guide RNA that targets a first protospacer sequence of the target cell genome) and wherein the carrier cell further comprises an *incF1* plasmid encoding an anti-target cell CRISPR-Cas system or a component thereof (eg, encoding a second crRNA or guide RNA that targets a secondprotospacer sequence of the target cell genome), the protospacers comprising different nucleotide sequences. For example, the protospacers are comprised by different genes of the target cell genome. For example the protospacers are comprised by one or more pathogenicity islands of the target cell genome. Optionally, the target cell is an

Enterobacteriaceae cell. Optionally, the carrier cell comprises a group of plasmids comprising 2, 3, 4, 5, 6 or more different types of plasmid, wherein each plasmid is capable of being conjugatively transferred into a target cell, wherein the plasmids encode different agents or different components of an antibacterial agent. For example, the plasmids encode different cRNAs or gRNAs that target different protospacers comprisesd by the target cell genome. For example, the group of plasmids comprises up to *n* different types of plasmid, wherein the plasmids are members of up to *n* different incompatibility groups, eg, groups selected from *IncFI, IncFII, IncFIll, IncFIV, IncFV, IncM, Inc9, InclO, Incl, IncA, IncB, IncC, IncH, IncIa, InclIc, IncI2, IncIy, IncJ, IncL, IncN, Inc2e, IncO, IncP, IncS, IncT* and *IncW*. For example, n=2, 3, 4, 5, 6, 7, 8, 9 or 10.

For example, the carrier cell comprises (i) a first plasmid that encodes a first type of CRISPR/Cas system that targets a first protospacer comprised by the target cell genome, or encodes a component of said system; and (ii) a second plasmid that encodes a second type of CRISPR/Cas system that targets a second protospacer comprised by the target cell genome, or encodes a component of said system, wherein the first and second types are different. For example, the first type is a Type I system, and the second type is a Type II system (eg, the first plasmid encodes a Cas3, Cascade and a crRNA or guide RNA that is operable with the Cas3 and Cascade in the target cell to modify the first protospacer; and the second plasmid encodes a Cas9 and a crRNA or guide RNA that is operable with the Cas9 in the target cell to modify the second protospacer). In an alternative, the Cas3 and Cascade are encoded by an endogenous target cell gene, wherein the first plasmid encodes the crRNA or guide RNA that is operable with the endogenous Cas3 and Cascade in the target cell to modify the first protospacer. In an alternative, the Cas9 is encoded by an endogenous target cell gene, wherein the second plasmid encodes the crRNA or guide RNA that is operable with the endogenous Cas9 in the target cell to modify the second protospacer. Optionally, the Cas3 and Cascade are encoded by endogenous genes of the target cell and the Cas9 is encoded by the second plasmid.

Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type I CRISPR/Cas system (or component thereof, eg, a Cas3 or a crRNA or a gRNA) and a second plasmid encoding a Type III CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type I CRISPR/Cas system (or component thereof) and a second plasmid encoding a Type IV CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type I CRISPR/Cas system (or component thereof) and a second plasmid encoding a Type V CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively

provides in an embodiment a first plasmid enocoding a Type I CRISPR/Cas system (or component thereof) and a second plasmid encoding a Type VI CRISPR/Cas system (or a component thereof).

Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type II CRISPR/Cas system (or component thereof, eg, a Cas9 or a crRNA or a gRNA) and a second plasmid encoding a Type III CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type II CRISPR/Cas system (or component thereof) and a second plasmid encoding a Type IV CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type II CRISPR/Cas system (or component thereof) and a second plasmid encoding a Type V CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type II CRISPR/Cas system (or component thereof) and a second plasmid encoding a Type VI CRISPR/Cas system (or a component thereof).

Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type V CRISPR/Cas system (or component thereof, eg, a Cas12a or a crRNA) and a second plasmid encoding a Type III CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type V CRISPR/Cas system (or component thereof) and a second plasmid encoding a Type IV CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type V CRISPR/Cas system (or component thereof) and a second plasmid encoding a Type V CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment a first plasmid enocoding a Type V CRISPR/Cas system (or component thereof) and a second plasmid encoding a Type VI CRISPR/Cas system (or a component thereof).

Instead of a Type I and Type II system, the invention alternatively provides in an embodiment first and second plasmids, each enocoding a Type I CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment first and second plasmids, each enocoding a Type II CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment first and second plasmids, each enocoding a Type III CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment first and second plasmids, each enocoding a Type IV CRISPR/Cas system (or a component thereof). Instead of a

Type I and Type II system, the invention alternatively provides in an embodiment first and second plasmids, each enocoding a Type V CRISPR/Cas system (or a component thereof). Instead of a Type I and Type II system, the invention alternatively provides in an embodiment first and second plasmids, each enocoding a Type VI CRISPR/Cas system (or a component thereof).

Optionally, the plasmids are members of different incompatibility groups, eg, groups selected from *IncFI, IncFII, IncFIII, IncFIV, IncFV, IncM, Inc9, InclO, Incl, IncA, IncB, IncC, IncH, IncIa, InclIc, IncI2, IncIy, IncJ, IncL, IncN, Inc2e, IncO, IncP, IncS, IncT* and *IncW*. In an example here, the target cell is an *Enterobacteriaceae* cell.

EMBODIMENTS

Thus, by way of example the invention provides the following Embodiments.

1. A carrier bacterial cell comprising a first episomal DNA, the DNA comprising a nucleic acid of interest (NSI) that encodes a protein of interest (POI) or RNA of interest (ROI) for expressing the POI or ROI in a target bacterial cell, the carrier cell being capable of conjugative transfer of the DNA into the target cell for expression therein of the POI, ROI or agent, wherein
   (a) the carrier cell comprises a second DNA which is different from the first DNA, wherein the second DNA comprises or encodes a first factor required for replication of the first DNA;
   (b) the first DNA does not comprise or encode said first factor, wherein the first DNA is non-self-replicative in the absence of the first factor, but is able to replicate in the carrier cell in the presence of the first factor provided by the second DNA;
   (c) wherein the carrier cell comprises genes encoding one or more conjugation factors sufficient to carry out conjugative transfer of the first DNA into a target bacterial cell.

In a first alternative, Embodiment 1 provides:-
A carrier bacterial cell comprising a first episomal DNA, the DNA comprising a nucleic acid of interest (NSI) that encodes a protein of interest (POI) or RNA of interest (ROI) for expressing the POI or ROI in a target bacterial cell, the carrier cell being capable of conjugative transfer of the DNA into the target cell for expression therein of the POI or ROI.

In a second alternative, Embodiment 1 provides:-

A carrier bacterial cell comprising a first episomal DNA, the DNA encoding an antibacterial agent that is toxic to a target bacterial cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into the target cell for expression therein of the agent.

In an example, the agent comprises a guided nuclease that is capable of recognizing and cutting a target nucleic acid sequence comprised by the target cell genome, wherein the target sequence is not comprised by the carrier cell. In this sense, therefore, the antibacterial agent is toxic to a target bacterial cell but is not toxic to the carrier cell.

For example, there is provided:-

A carrier bacterial cell comprising a first episomal DNA, the DNA encoding an antibacterial agent that is toxic to a target bacterial cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into the target cell for expression therein of the agent, wherein the agent is a component of a CRISPR/Cas system that is operable in the target cell to modify a target nucleic acid sequence comprised by the target cell genome (eg, comprised by the target cell chromosome).

Another example provides:-

A carrier bacterial cell comprising a first episomal DNA, the DNA encoding an antibacterial agent that is toxic to a target bacterial cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into the target cell for expression therein of the agent, wherein

(a) the carrier cell comprises a second DNA which is different from the first DNA, wherein the second DNA comprises or encodes a first factor required for replication of the first DNA;

(b) the first DNA does not comprise or encode said first factor, wherein the first DNA is non-self-replicative in the absence of the first factor, but is able to replicate in the carrier cell in the presence of the first factor provided by the second DNA;

(c) wherein the carrier cell comprises genes encoding one or more conjugation factors sufficient to carry out conjugative transfer of the first DNA into a target bacterial cell.

An example also provides:-

A carrier bacterial cell comprising a plasmid comprising a first DNA, the DNA encoding a guided nuclease that is capable of recognising and modifying a target nucleic acid sequence, the carrier cell being capable of conjugative transfer of the plasmid into the target cell for expression therein of the nuclease, wherein the target sequence is comprised by an endogenous chromosome or episome of the target cell but is not comprised by the carrier cell, wherein the nuclease is capable of modifying (eg,

cutting) the chromosome or episome to kill the target cell or inhibit the growth or proliferation of the target cell.

Optionally, the nuclease is a Cas nuclease, meganuclease, zinc finger nuclease or TALEN.

Optionally, the nuclease is a Cas nuclease of a Type I, II, III, IV or V CRISPR system.

An example also provides:-

A carrier bacterial cell comprising a plasmid comprising a first DNA, the DNA encoding a component of a CRISPR/Cas system that is capable of recognising and modifying a target nucleic acid sequence, the carrier cell being capable of conjugative transfer of the plasmid into the target cell for expression therein of the component, whereby the expressed component forms part of a said system in the target cell, wherein the target sequence is comprised by an endogenous chromosome or episome of the target cell but is not comprised by the carrier cell, wherein the system is capable of modifying (eg, cutting) the chromosome or episome to kill the target cell or inhibit the growth or proliferation of the target cell.

In an aspect, the component is a guide RNA or crRNA that is capable of hybridising to the target sequence of the target cell. They system may be a Type I, II, III, IV or V CRISPR system.

Optionally,

(a) the carrier cell comprises a second DNA which is different from the first DNA, wherein the second DNA comprises or encodes a first factor required for replication of the first DNA;

(b) the first DNA does not comprise or encode said first factor, wherein the first DNA is non-self-replicative in the absence of the first factor, but is able to replicate in the carrier cell in the presence of the first factor provided by the second DNA;

(c) wherein the carrier cell comprises genes encoding one or more conjugation factors sufficient to carry out conjugative transfer of the first DNA into a target bacterial cell.

The second DNA may be comprised by a chromosome or episome (eg, plasmid) of the carrier cell.

Any method of the invention may use carrier cell(s) of any of these examples.

Advantageously, the cell is for treating or preventing a target cell infection in a human or an animal subject (eg, a chicken, cow, pig, fish or shellfish). Advantageously, the carrier cell is a cell of a species that is probiotic to said subject or is probioitic to humans or animals (eg, chickens). For example, the carrier cell is a probiotic *E coli* cell. For example, the carrier cell is a probiotic *Bacillus* cell. In an example, the target cell is a cell of a species that is pathogenic to said subject, or is pathogenic to humans or aniumals (eg, chickens). Advantageously, the first DNA encodes one or more guide RNAs or one or more crRNAs that are capable of hybridizing in the target cell to respective target nucleic acid sequence(s), wherein the target sequence(s) are comprised by an

endogenous chromosome and/or endogenous episome of the target cell. For example, the first DNA encodes 2, 3, 4, 5, 6, 7, 7, 9, or 10 (or more than 10) different gRNAs or different crRNAs that hybridise to a respective target sequence, wherein the target sequences are different from each other. For example, 3 different gRNAs or crRNAs are encoded by the first DNA. For example, 2 different gRNAs or crRNAs are encoded by the first DNA. For example, 3 different gRNAs or crRNAs are encoded by the first DNA. For example, 4 different gRNAs or crRNAs are encoded by the first DNA. For example, 3 different gRNAs or crRNAs are encoded by the first DNA. For example, 5 different gRNAs or crRNAs are encoded by the first DNA. For example, 6 different gRNAs or crRNAs are encoded by the first DNA. For example, 7 different gRNAs or crRNAs are encoded by the first DNA. For example, 8 different gRNAs or crRNAs are encoded by the first DNA. For example, 9 different gRNAs or crRNAs are encoded by the first DNA. For example, 10 different gRNAs or crRNAs are encoded by the first DNA. For example, 11 different gRNAs or crRNAs are encoded by the first DNA. For example, 12 different gRNAs or crRNAs are encoded by the first DNA. For example, 13 different gRNAs or crRNAs are encoded by the first DNA. In an example, the target cells are *Salmonella* cells (eg, wherein the subject is a chicken). In an example, the target cells are *E coli* cells. In an example, the target cells are *Campylobacter* cells (eg, wherein the subject is a chicken). In an example, the target cells are *Edwardsiella* cells (eg, wherein the subject is a fish or shellfish, eg, a catfish or a shrimp or prawn). In an example, the target cells are *E coli* cells.

In an alternative herein, the carrier and target cells are archaeal cells. In an alternative herein, the carrier and target cells are yeast cells and "conjugation" is to be read instead as referring to yeast "mating" and, eg, the second DNA is comprised by a chromosome of the carrier yeast cell.

In a preferred example, the NOI encodes an antibacterial agent that is toxic to a target bacterial cell but is not toxic to the carrier cell. In an example, the POI is an antibiotic agent, an antibody, an antibody chain or an antibody variable domain. In an example, the ROI is a guide RNA or a crRNA that is operable in the target cell with a cognate Cas (eg, a Cas nuclease to target and cut a protospacer sequence comprised by a chromosome or episome of the target cell). In an example the RNA is a siRNA that is capable of hybridizing to an endogenous target nucleic acid sequence of the target cell to silence transcription and/or translation thereof.

The the carrier cell comprises a chromosome or second episomal DNA which is different from the first DNA. Thus, for example the second DNA is comprised by a chromosome of the carrier cell. In an other example, the second DNA is comprised by a plasmid of the carrier cell.

2. The carrier cell of Embodiment 1, wherein

   (d) the first DNA is devoid of a component required for conjugative transfer of the first DNA into a target bacterial cell; and

(e) the carrier cell comprises said component, wherein the component is comprised by or encoded by the second DNA or a third DNA comprised by the carrier cell.

The third DNA may be comprised by a chromosome or episome (eg, plasmid). Where explicitly recited in Embodiments 2 onwards, the component is referring to the component required for conjugative transfer, as the context makes clear.

3. The carrier cell of Embodiment 2, wherein the component is comprised by a Mpf or Dtr module. For example, the module is a RK2, RP4 or R6K tra module. Optionally, the component is comprised by a Mpf (mating pair formation) module, eg, a RK2 or RP4 tra1 module or a homologue thereof. Optionally, the component is comprised by a Dtr (DNA-transfer replication) module, eg, a RK2 or RP4 tra2 module or a homologue thereof.

4. The carrier cell of Embodiment 2, wherein the component is encoded by an operon of a Mpf (eg, tra1) or Dtr (eg, tra2) module .

For example, the module is a RK2, RP4 or R6K tra module. Optionally, the module is a Mpf (mating pair formation) module, eg, a RK2 or RP4 tra1 module or a homologue thereof. Optionally, the module is a Dtr (DNA-transfer replication) module, eg, a RK2 or RP4 tra2 module or a homologue thereof.

The Mpf operon of RP4 or RK2 is the tra2 operon (comprising the trb genes trbBCDEFGHIJKL) together with gene traF. The gene traF is comprised in RK2 or RP4 in a tra1 operon (along with traJXIHG). Thus, in an embodiment, the component is a gene selected from trbB, trbC, trbD, trbE, trbF, trbG, trbH, trbI, trbJ, trbK, trbL and traF. In an embodiment the first DNA is devoid of two or more genes selected from RP4 trbB, trbC, trbD, trbE, trbF, trbG, trbH, trbI, trbJ, trbK, trbL and traF or homologues thereof. In an embodiment the first DNA is devoid of two or more genes selected from RK2 trbB, trbC, trbD, trbE, trbF, trbG, trbH, trbI, trbJ, trbK, trbL and traF or homologues thereof. In an example, the component is a traK, traL or traM gene. In an embodiment the first DNA is devoid of two or more genes selected from RP4 traK, traL and traM or homologues thereof. In an embodiment the first DNA is devoid of two or more genes selected from RK2 traK, traL and traM or homologues thereof. Optionally, in these embodiments the first DNA is comprised by a RP4 or RK2-type plasmid.

R6K Mpf is encoded by the sltX1tivB1B2B3-4B5B6B7B8B9B10B11 operon that also includes clpX1 of the Dtr module. Thus, in an embodiment, the component is a gene comprises by the RK6 sltX1tivB1B2B3-4B5B6B7B8B9B10B11 operon. In an embodiment, the the component is clpX1 of the RK6 Dtr module. In an embodiment, the component is a gene selected from clpX1 and

dtrX1rlxX1. In an embodiment, the component is a gene selected from a RK6 clpX1 and dtrX1rlxX1 or homologues thereof. Optionally, in these embodiments the first DNA is comprised by a RK6-type plasmid.

The carrier cell of any preceding Embodiment, wherein the carrier cell chromosome and/or an episome of the carrier cell (other than an episome comprising the first DNA) comprises an expressible tra1 and/or tra2 module or a homologue thereof.
Any episome herein may be a plasmid.

5. The carrier cell of any preceding Embodiment, wherein the carrier cell chromosome and/or an episome of the carrier cell (other than an episome comprising the first DNA) comprises an expressible operon of a tra1 and/or tra2 module or a homologue thereof.

6. The carrier cell of any one of Embodiments 3 to 6, wherein the component is tra1 component.

7. The carrier cell of any one of Embodiments 3 to 6, wherein the component is tra2 component.

8. The carrier cell of any one of Embodiments 2 to 7, wherein the carrier cell chromosome encodes the first factor and comprises said component.

9. The carrier cell of any preceding Embodiment, wherein the first factor is a *rep* protein, optionally wherein the protein is encoded by *pir* or *trfA* or a homologue thereof.

10. The carrier cell of any preceding Embodiment, wherein the first DNA is comprised by a RK2 or R6K plasmid.

11. The carrier cell of any preceding Embodiment, wherein the first DNA comprises an *oriV* of a RK2 or R6K plasmid, or a homologue thereof.

12. The carrier cell of any preceding Embodiment, wherein the first DNA comprises an *oriT* of a RK2 or R6K plasmid, or a homologue thereof.

13. The carrier cell of any preceding Embodiment, wherein the first DNA is comprised by a plasmid.

14. The carrier cell of any preceding Embodiment, wherein the second DNA is comprised by a plasmid or is comprised by a chromosome of the carrier cell.

15. The carrier cell of any preceding Embodiment, wherein the agent comprises one or more components of a CRISPR/Cas system that is operable in the target cell to cut a protospacer sequence comprised by the target cell, eg, wherein the protospacer sequence is comprised by the cell chromosome.

In an embodiment, the cutting herein kills the target cell. In another embodiment, the cutting inhibits the growth or proliferation of the target cell.

16. The carrier cell of any preceding Embodiment, wherein the agent encodes a guide RNA or crRNA of a CRISPR/Cas system that is operable with a Cas nuclease in the target cell to cut a protospacer sequence comprised by the target cell, eg, wherein the protospacer sequence is comprised by the cell chromosome.

In an example, the target cell is a *Salmonella* cell and the protospacer is comprised by a *pipA, pipB, pipC, hilA, sicP, mart* or *sopB* gene. In an example, the protospacer is comprised by a gene that is a homologue or orthologue of a *Salmonella sicP, sseF, pipA, pipB, pipC, hilA, sicP, mart* or *sopB* gene.

17. The carrier cell of any preceding Embodiment, wherein the first DNA comprises a gene that encodes a product, wherein the product is essential for survival or proliferation of the carrier cell when in an environment that is devoid of the product, wherein the carrier cell chromosome does not comprise an expressible gene encoding the product and optionally the first DNA is the only episomal DNA comprised by the carrier cell that encodes the product.

18. The carrier cell of Embodiment 17, wherein the gene is selected from an *aroA, argH, hisD, leuB, lysA, metB, proC, thrC, pheA, tyrA, trpC* and *pflA* gene; or wherein the gene is an anti-toxin gene and optionally the first DNA encodes a cognate toxin.

19. The carrier cell of any preceding Embodiment, wherein the carrier cell is an *E coli* (eg, Nissle, F18 or S17 *E coli* strain), *Bacillus* (eg, *B subtilis*), *Enterococcus* or *Lactobacillus* cell.

Optionally, the carrier cell is a cell of a human, chicken pig, sheep, cow, fish (eg, catfish or salmon) or shellfish (eg, shrimp or lobster) commensal bacterial strain (eg, a commensal *E coli* strain).

20. The carrier cell of any preceding Embodiment, wherein the carrier cell is for administration to a microbiota of a human or animal subject for medical use.

For example, the medical use is for treating or preventing a disease disclosed herein. For example, the medical use is for treating or preventing a condition disclosed herein.

21. The carrier of Embodiment 20, wherein the medical use is for the treatment or prevention of a disease or condition mediated by said target cells.

22. The carrier of any one of Embodiments 1 to 20 for administration to an animal for enhancing growth or weight of the animal.

In alternative, the administration is to a human for enhancing the growth or weight of the human. Optionally, the enhancing is not a medical therapy. Optionally, the enhancing is a medical therapy.

23. The carrier of any one of Embodiments 20 to 22, wherein the use comprises the administration of a plurality of carrier cells to a microbiota (eg, a gut microbiota) of the subject, wherein the microbiota comprises target cells and first DNA is transferred into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the subject or reducing the growth or proliferation of target cells.

24. A DNA encoding an antibacterial agent that is toxic to a target bacterial cell but is not toxic to a carrier cell, wherein the first DNA comprises an origin of replication but does not comprise or encode a first factor required for replication of the first DNA, and wherein the first DNA comprises an origin of transfer but is devoid of a component required for conjugative transfer of the first DNA into a target bacterial cell, wherein when in the presence of the component the DNA is capable of conjugative transfer into the target cell.

Thus, when the DNA is in a carrier cell, the DNA is capable of conjugative transfer into a target cell.

25. The DNA of Embodiment 24, wherein the DNA is according to the DNA recited in any one of Embodiments 1 to 23.

26. The DNA of Embodiment 24 or 25, wherein the cells are according to the cells recited in any one of Embodiments 1 to 23.

27. A method for enhancing growth or weight of an animal subject (eg, a chicken), wherein the method comprises the administration of a plurality of carrier cells according to any one of Embodiments 1 to 23 to a microbiota of the subject, wherein the microbiota comprises target cells and first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells (eg, *Salmonella* cells) in the subject or reducing the growth or proliferation of target cells.

In alternative, the administration is to a human for enhancing the growth or weight of the human. Optionally, the enhancing is not a medical therapy. Optionally, the enhancing is a medical therapy.

28. A method for enhancing growth or weight of a plant (eg, a tomato plant), wherein the method comprises the administration of a plurality of carrier cells according to any one of Embodiments 1 to 23 to a microbiota of the plant, wherein the microbiota comprises target cells and first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells (eg, *Pseudomonas* cells) in the plant or reducing the growth or proliferation of target cells.

The plant may be any plant disclosed herein. For example a plant herein in any configuration or embodiment of the invention is selected from a tomato plant, a potato plant, a wheat plant, a corn plant, a maize plant, an apple tree, a bean-producing plant, a pea plant, a beetroot plant, a stone fruit plant, a barley plant, a hop plant and a grass. For example, the plant is a tree, eg, palm, a horse chestnut tree, a pine tree, an oak tree or a hardwood tree. For example the plant is a plant that produces fruit selected from strawberries, raspberries, blackberries, reducrrants, kiwi fruit, bananas, apples, apricots, avoocados, cherries, oranges, clementines, satsumas, grapefruits, plus, dates, figs, limes, lemons, melons, mangos, pears, olives or grapes. Optionally, the plant is a dicotyledon. Optionally, the plant is a flowering plant. Optionally, the plant is a monocotyledon.

In any configuration, embodiment or example herein, the target bacteria are *P syringae* bacteria (eg, comprised by a plant). *Pseudomonas syringae pv. syringae* is a common plant-associated bacterium

that causes diseases of both monocot and dicot plants worldwide. In an example the targt bacteria are *P syringae* bacteria of a pathovar selected from *P. s.* pv. *aceris*, *P. s.* pv. *aptata*, *P. s.* pv. *atrofaciens*, *P. s.* pv. *dysoxylis*, *P. s.* pv. *japonica*, *P. s.* pv. *lapsa*, *P. s.* pv. *panici*, *P. s.* pv. *papulans*, *P. s.* pv. *pisi*, *P. s.* pv. *syringae* and *P. s.* pv. *morsprunorum.*

- *P. s.* pv. *aceris* attacks maple Acer species.
- *P. s.* pv. *actinidiae* attacks kiwifruit Actinidia deliciosa.
- *P. s.* pv. *aesculi* attacks horse chestnut Aesculus hippocastanum, causing bleeding canker.
- *P. s.* pv. *aptata* attacks beets Beta vulgaris.
- *P. s.* pv. *atrofaciens* attacks wheat Triticum aestivum.
- *P. s.* pv. *dysoxylis* attacks the kohekohe tree Dysoxylum spectabile.
- *P. s.* pv. *japonica* attacks barley Hordeum vulgare.
- *P. s.* pv. *lapsa* attacks wheat *Triticum aestivum.*
- *P. s.* pv. *panici* attacks Panicum grass species.
- *P. s.* pv. *papulans* attacks crabapple Malus sylvestris species.
- *P. s.* pv. *phaseolicola* causes halo blight of beans.
- *P. s.* pv. *pisi* attacks peas Pisum sativum.
- *P. s.* pv. *syringae* attacks Syringa, Prunus, and Phaseolus species.
- *P. s.* pv. *glycinea* attacks soybean, causing bacterial blight of soybean.

In an example, the target bacteria are *P syringae* selected from a serovar recited in a bullet point in the immediately preceding paragraph and the bacteria are comprised by a plant also mentioned in that bullet point.

In an example, the weight is dry weight. For example, the method is for increasing dry weight (eg, within 1 or 2 weeks of said administration). Optionally, the increase is an increase of at least 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% compared to a control plant of the same species or strain to which the administration if carrier cells has not taken place, wherein all plants are kept under the same environmental conditions. For example, such an increase is within 1, 2, 3, 4, 5, 6, or 8 weeks following the first administration of the carrier cells. In an example, the method is for increasing the dry weight of a leaf and/or fruit of the plant, such as a tomato plant.

In an example, the weight is wet weight. For example, the method is for increasing wet weight (eg, within 1 or 2 weeks of said administration). Optionally, the increase is an increase of at least 5, 10,

11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% compared to a control plant of the same species or strain to which the administration if carrier cells has not taken place, wherein all plants are kept under the same environmental conditions. For example, such an increase is within 1, 2, 3, 4, 5, 6, or 8 weeks following the first administration of the carrier cells. In an example, the method is for increasing the dry weight of a leaf and/or fruit of the plant, such as a tomato plant.

29. The method of Embodiment 28, wherein the microbiota is comprised by a leaf, trunk, root or stem of the plant.

In an example, in any configuration or embodiment herein the target bacteria (or taraget cell) is comprised by a microbiota of a plant. In an example, the microbiota is comprised by a leaf. In an example, the microbiota is comprised by a xylem. In an example, the microbiota is comprised by a phloem. In an example, the microbiota is comprised by a root. In an example, the microbiota is comprised by a tuber. In an example, the microbiota is comprised by a bulb. In an example, the microbiota is comprised by a seed. In an example, the microbiota is comprised by an exocarp, epicarp, mesocarp or endocarp. In an example, the microbiota is comprised by a fruit, eg, a simple fruits; aggregate fruits; or multiple fruits. In an example, the microbiota is comprised by a seed or embryo, eg, by a seed coat; a seed leaf; cotyledons; or a radicle. In an example, the microbiota is comprised by a flower, eg, comprised by a peduncle; sepal: petals; stamen; filament; anther or pistil. In an example, the microbiota is comprised by a root; eg, a tap root system, or a fibrous root system. In an example, the microbiota is comprised by a leaf or leaves, eg, comprised by a leaf blade, petiole or stipule. In an example, the microbiota is comprised by a stem, eg, comprised by bark, epidermis, phloem, cambium, xylem or pith.

30. A method for reducing a biofilm comprised by a subject or comprised on a surface, wherein the biofilm comprises target cells (eg, *Pseudomonas* cells), wherein the method comprises the administration of a plurality of carrier cells according to any one of Embodiments 1 to 23 to the biofilm, wherein first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the biofilm or reducing the growth or proliferation of target cells.

In an example "reducing a biofilm" comprises reducing the coverage area of the biofilm. In an example "reducing a biofilm" comprises reducing the proliferation of the biofilm. In an example "reducing a biofilm" comprises reducing the durability of the biofilm. In an example "reducing a biofilm" comprises reducing the spread of the biofilm (eg, in or on the subject, eg, spread to the

environment containing the subject).

31. The method of Embodiment 30, wherein the subject is a human or animal.

For example, the biofilm is comprised by a lung of the subject, eg, wherein the target cells are *Pseudomonas* (eg, *P aeruginosa*) cells. This may be useful wherein the subject is a human suffering from a lung disease or condition, such as pneumonia or cystic fibrosis.
For example, the biofilm is comprised by an animal or human organ disclosed herein. For example, the biofilm is comprised by a microbiota of a human or animal disclosed herein.

32. The method of Embodiment 30, wherein the subject is a plant (eg, any plant disclosed herein).

Optionally, in this Embodiment the target cells are *Pseudomonas syringae* cells.

33. The method of Embodiment 31 or 32, wherein the biofilm is comprised by a leaf, trunk, root or stem of the plant.

In an example, in any configuration or embodiment herein the target bacteria (or taraget cell) is comprised by a biofilm of a plant. In an example, the biofilm is comprised by a leaf. In an example, the biofilm is comprised by a xylem. In an example, the biofilm is comprised by a phloem. In an example, the biofilm is comprised by a root. In an example, the biofilm is comprised by a tuber. In an example, the biofilm is comprised by a bulb. In an example, the biofilm is comprised by a seed. In an example, the biofilm is comprised by an exocarp, epicarp, mesocarp or endocarp. In an example, the biofilm is comprised by a fruit, eg, a simple fruits; aggregate fruits; or multiple fruits. In an example, the biofilm is comprised by a seed or embryo, eg, by a seed coat; a seed leaf; cotyledons; or a radicle. In an example, the biofilm is comprised by a flower, eg, comprised by a peduncle; sepal: petals; stamen; filament; anther or pistil. In an example, the biofilm is comprised by a root; eg, a tap root system, or a fibrous root system. In an example, the biofilm is comprised by a leaf or leaves, eg, comprised by a leaf blade, petiole or stipule. In an example, the biofilm is comprised by a stem, eg, comprised by bark, epidermis, phloem, cambium, xylem or pith.

34. The method of any one of Embodiments 30 to 33 (eg, 30), wherein the surface is a surface *ex vivo*, such as a surface comprised by a domestic or industrial apparatus or container.

35. A method of replicating a first DNA to produce a plurality of copies of said DNA, the method comprising culturing a plurality of carrier bacterial cells according to any one of Embodiments 1 to 23, wherein the first DNA is replicated in the cells.

Optionally, the method further comprises isolating first DNA after said culturing. The skilled addresse is familiar with techniques and conditions for culturing cells that can be used.

36. The method of Embodiment 35, comprising
    (a) obtaining a sample of the carrier cells after culturing;
    (b) contacting the sample of carrier cells with a plurality of target bacterial cells to allow conjugation between carrier cells and target cells; and
    (c) allowing copies of first DNA to be transferred by conjugative transfer from carrier cells to target cells, wherein the antibacterial agent is provided in target cells and target cells are killed or the growth or proliferation of target cells is reduced;
    (d) wherein the first DNA is not replicable in the target cells.

For example, the first DNA is not substantially replicable in the target cells, eg, less than 10, 5, 4, 3, 2 or 1% replication).

The first DNA is not (or not substantially) replicable in the target cells due to the absence of the first factor or sequence encoding it in the target cells.

37. A method of killing a plurality of target bacterial cells or reducing the growth or proliferation thereof, the method comprising
    (a) obtaining a sample of the carrier cells according to any one of Embodiments 1 to 23 or obtainable by the method of Embodiment 35;
    (b) contacting the sample of carrier cells with the plurality of target bacterial cells to allow conjugation between carrier cells and target cells; and
    (c) allowing copies of first DNA to be transferred by conjugative transfer from carrier cells to target cells, wherein the antibacterial agent is provided in target cells and target cells are killed or the growth or proliferation of target cells is reduced;
    (d) wherein the first DNA is not (or not substantially) replicable in the target cells.

38. The method of Embodiment 36 or 37, wherein the target cells are comprised by a biofilm, eg, a biofilm as disclosed herein.

39. A method for containing the action of an antibacterial agent in an environment, wherein the agent is toxic to target bacterial cells, the method comprising carrying out the method of Embodiment 37 or 38 and the agent is said agent recited of the Embodiment.

40. The method of Embodiment 39, wherein the environment is comprised by a human or animal subject and the target cells are comprised by a biofilm of the subject, wherein the method comprises administering the sample of carrier cells to the biofilm of the subject, wherein the carrier cells are contacted with the target cells in step (b), wherein the method is a contained method for treating or preventing a disease or condition mediated by target cells in the subject.

41. The carrier cell, DNA or method of any preceding Embodiment, wherein the target bacteria are *Salmonella, Pseudomonas, Escherichia, Klebsiella, Campylobacter, Helicobacter, Acinetobacter, Enterobacteriacea, Clostridium, Staphylococcus* or *Streptococcus* bacteria.

42. The carrier cell, DNA or method of any preceding Embodiment, wherein the target bacteria are *Salmonella enterica* bacteria.

For example, the target bacteria are selected from the group consisting of *Salmonella enterica* subsp. *enterica*, serovars Typhimurium, Enteritidis, Virchow, Montevideo, Hadar and Binza.

43. The carrier cell, DNA or method of any one of Embodiments 1 to 41, wherein the target bacteria are *Pseudomonas* (eg, *P syringae* or *P aeruginosa)* bacteria.

44. The carrier cell or method of any one of Embodiments 1 to 41, wherein the target bacteria are *E coli* bacteria.

Optionally, the target bacteria are enterohemorrhagic *E. coli* (EHEC), *E. coli* Serotype O157:H7 or Shiga-toxin producing *E. coli (*STEC)). In an example, the taraget bacteria are selected from

- Shiga toxin-producing *E. coli* (STEC) (STEC may also be referred to as Verocytotoxin-producing *E. coli* (VTEC);
- Enterohemorrhagic *E. coli* (EHEC) (this pathotype is the one most commonly heard about in the news in association with foodborne outbreaks);

- Enterotoxigenic *E. coli* (ETEC);
- Enteropathogenic *E. coli* (EPEC);
- Enteroaggregative *E. coli* (EAEC);
- Enteroinvasive *E. coli* (EIEC); and
- Diffusely adherent *E. coli* (DAEC).

Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 is a human pathogen responsible for outbreaks of bloody diarrhoea and haemolytic uremic syndrome (HUS) worldwide. Conventional antimicrobials trigger an SOS response in EHEC that promotes the release of the potent Shiga toxin that is responsible for much of the morbidity and mortality associated with EHEC infection. Cattle are a natural reservoir of EHEC, and approximately 75% of EHEC outbreaks are linked to the consumption of contaminated bovine-derived products. EHEC causes disease in humans but is asymptomatic in adult ruminants. Characteristics of *E. coli* serotype O157:H7 (EHEC) infection includes abdominal cramps and bloody diarrhoea, as well as the life-threatening complication haemolytic uremic syndrome (HUS). Currently there is a need for a treatment for EHEC infections (Goldwater and Bettelheim, 2012). The use of conventional antibiotics exacerbates Shiga toxin-mediated cytotoxicity. In an epidemiology study conducted by the Centers for Disease Control and Prevention, patients treated with antibiotics for EHEC enteritis had a higher risk of developing HUS (Slutsker et al., 1998). Additional studies support the contraindication of antibiotics in EHEC infection; children on antibiotic therapy for hemorrhagic colitis associated with EHEC had an increased chance of developing HUS (Wong et al., 2000; Zimmerhackl, 2000; Safdar et al., 2002; Tarr et al., 2005). Conventional antibiotics promote Shiga toxin production by enhancing the replication and expression of *stx* genes that are encoded within a chromosomally integrated lambdoid prophage genome. The approach of some configurations of present invention rely on nuclease cutting. *Stx* induction also promotes phage-mediated lysis of the EHEC cell envelope, allowing for the release and dissemination of Shiga toxin into the environment (Karch et al., 1999; Matsushiro et al., 1999; Wagner et al., 2002). Thus, advantageously, these configurations of the invention provide alternative means for treating EHEC in human and animal subjects. This is exemplified below with surprising results on the speed and duration of anti-EHEC action produced by nuclease action (as opposed to conventional antibiotic action).

In an example, the subject (eg, a human or animal) is suffering from or at risk of haemolytic uremic syndrome (HUS), eg, the subject is suffering from an E coli infection, such as an EHEC *E coli* infection.

45. A pharmaceutical composition, livestock growth promoting composition, soil improver, herbicide, plant fertilizer, food or food ingredient sterilizing composition, dental composition, personal hygiene composition or disinfectant composition (eg, for domestic or industrial use) comprising a plurality of carrier cells according to any one of Embodiments 1 to 23.

Herein, a carrier cell is, eg, a probiotic cell for administration to a human or animal subject. For example, the carrier cell is commensal in a microbiome (eg, gut or blood microbiome) of a human or animal subject, wherein the carrier is for administration to the subject. In an example, a carrier cell is a bacterial cell (and optionally the target cell is a bacterial cell). In an example, a carrier cell is an archaeal cell (and optionally the target cell is an archaeal cell)

Optionally, the carrier cell is a gram-positive bacterial cell and the target cell is a gram-positive bacterial cell.

Optionally, the carrier cell is a gram-positive bacterial cell and the target cell is a gram-negative bacterial cell.

Optionally, the carrier cell is a gram-negative bacterial cell and the target cell is a gram-positive bacterial cell.

Optionally, the carrier cell is a gram-negative bacterial cell and the target cell is a gram-negative bacterial cell.

Optionally, the carrier cell is a *Bacillus* bacterial cell and the target cell is a gram-positive bacterial cell.

Optionally, the carrier cell is a *Bacillus* bacterial cell and the target cell is a gram-netative bacterial cell.

Optionally, the carrier cell is a *Bacillus* bacterial cell and the target cell is a *Salmonella* bacterial cell.

Optionally, the carrier cell is a *Bacillus* bacterial cell and the target cell is an *E coli* bacterial cell.

Optionally, the carrier cell is an *E coli* bacterial cell and the target cell is a *Pseudomonas* bacterial cell.

Optionally, the carrier cell is an *E coli* bacterial cell and the target cell is a gram-positive bacterial cell.

Optionally, the carrier cell is an *E coli* bacterial cell and the target cell is a gram-netative bacterial cell.

Optionally, the carrier cell is an *E coli* bacterial cell and the target cell is a *Salmonella* bacterial cell.

Optionally, the carrier cell is an *E coli* bacterial cell and the target cell is an *E coli* bacterial cell.

Optionally, the carrier cell is an *E coli* bacterial cell and the target cell is a *Pseudomonas* bacterial cell.

A *Bacillus* cell herein is optionally a *B subtilis* cell.

Optionally, the carrier cell is a probiotic or commensal *E coli* bacterial cell for administration to a human or animal subject. Optionally, the carrier cell is a probiotic or commensal *Bacillus* bacterial cell for administration to a human or animal subject.

Herein, optionally the first DNA is comprised by a plasmid, eg, a closed circular DNA.

In an embodiment, in an example the first DNA is dsDNA. In an embodiment, in an example the first DNA is ssDNA.

In an alternative configuration, the first DNA is instead a first RNA.

Optionally, the target cell is a *Salmonella* cell (eg, wherein the carrier cell is an *E coli* cell), eg, a *Salmonella enterica* subsp. *enterica*, eg, a *Salmonella enterica* subsp. *enterica* serovar Typhimurium, Enteritidis, Virchow, Montevideo, Hadar or Binza.

For example, the target bacteria are selected from the group consisting of *S enterica; S typhimurium; P aeruginosa; E coli; K pneumoniae; C jujeni; H pylori; A baumanii; C difficile; S aureus; S pyogenes* or *S thermophilus.*

In an example, the target cell is a cell of a species that causes nosocomial infection in humans.

Optionally, the target cell is comprised by an animal (eg, poultry animal (such as chicken), swine, cow, fish (eg, catfish or salmon) or shellfish (eg, prawn or lobster)) microbiome. Optionally, the microbiome is a gut microbiome. For example, the target cell is a *Salmonella* cell comprised by a chicken gut biofilm. For example, the target cell is a *Salmonella* cell comprised by a chicken gut biofilm sample *ex vivo*.

In an embodiment, the first DNA comprises a bacterial oriV and/or an oriT. In an embodiment, the first DNA is comprised by a plasmid, wherein the plasmid comprises and oriV and/or an oriT.

The first factor may be a protein or RNA. For example, the first factor is *pir* or *trfA*. In an example the first factor is operable with an oriV comprised by the first DNA for replication thereof.

In an embodiment, the first DNA is comprised by a plasmid, wherein the plasmid comprises an oriV and does not encode any replication protein (eg, *pir* or *trfA*) that is operable with the oriV to initiate replication of the plasmid.

In an embodiment, the first DNA is devoid of a component required for conjugative transfer of the first DNA into a target bacterial cell. Optionally, the component is a protein. Optionally, the component is a RNA. Optionally, the component is a tra1 component. Optionally, the component is a tra2 component. In an example, the carrier cell comprises said component, wherein the component is comprised by or encoded by the carrier cell chromosome, the second DNA or a third DNA in the carrier cell. Preferably, the component is comprised by or encoded by the carrier cell chromosome.

Optionally, the component required for conjugative transfer is a RP4 plasmid component, eg, a component of a RP4 tra module (eg, tra1 or tra2 module).

Optionally, the component required for conjugative transfer is a RK2 plasmid component, eg, a component of a RK2 tra module (eg, tra1 or tra2 module).

Optionally, the component required for conjugative transfer is a R6K plasmid component, eg, a component of a R6K tra module (eg, tra1 or tra2 module).

Optionally, the first DNA is comprised by a plasmid that does not comprise an antibiotic resistance marker gene and/or a plasmid addiction system gene. As explained in the Examples section, this may be advantageous in the rare case of an identical IncP plasmid being present in the target cell that will provide trfA (or other component required for conjugation where that component is not encoded by the plasmid comprising the first DNA), both plasmids will compete for the available TrfA (or other component), resulting in loss of one plasmid, and thus will be quickly lost from any offspring target cells.

Optionally, the plasmid comprising the first DNA further comprises an anti-restriction gene encoding a product for inhibiting a restriction enzyme of the target cell (eg, an Type I restriction enzyme). as anti-restriction genes that inhibit Type I restriction enzymes (eg, the anti-restriction gene product is an ocr of T7, klcA of RK2, ard or ardB).

Additionally or alternatively, the plasmid comprises a gene encoding an essential component of a type IV secretion system, wherein the chromosome of the carrier cell comprises genes encoding the remainder of the secretion system, wherein the essential component is required for conjugative transfer of the plasmid from the carrier cell to the target cell. For example, with the exception of a tra1 or tra2 gene encoding the essential component, the chromosome comprises all of the remainder of traKLM, traJXIHGF genes of RK2 tra1 and all of the remainder of trbBCEFGHJL genes of RK2 tra2 (preferably when the plasmid is a RK2-type plasmid). In an alternative example, with the exception of a gene encoding the essential component, the chromosome comprises all of the reaminder of RK6 homologues of traKLM, traJXIHGF genes of RK2 tra1 and all of the remainder of RK6 homologues of trbBCEFGHJL genes of RK2 tra2 (preferably when the plasmid is a RK6-type plasmid). In an alternative example, with the exception of a gene encoding the essential component, the chromosome comprises all of the reaminder of RP4 homologues of traKLM, traJXIHGF genes of RK2 tra1 and all of the remainder of RP4 homologues of trbBCEFGHJL genes of RK2 tra2 (preferably when the plasmid is a RP4-type plasmid).

For example, with the exception of a tra1 or tra2 gene encoding the essential component, the chromosome comprises all of the remainder of a DNA fragment of the RK2 plasmid from traF to traM and all of the remainder of a DNA fragment of the RK2 plasmid from trbB to trK (preferably when the plasmid is a RK2-type plasmid). For example, with the exception of a tra1 or tra2 gene encoding the essential component, the chromosome comprises all of the remainder of the RK2 plasmid genes from traF to traM and all of the remainder of the RK2 plasmid genes from trbB to trK (preferably when the plasmid is a RK2-type plasmid). For example, the chromosome comprises (in the following

order 5' to 3', or 3' to 5') the following RK2 tra1 genes: traFGHIXJKLM, or traKLM, or traJXIHGF. For example, the chromosome comprises (in the following order 5' to 3', or 3' to 5') the following RK2 tra2 genes: trbBCDEFGHIJKL or trbBCEFGHJL.

For example, with the exception of a gene encoding the essential component, the chromosome comprises all of the remainder of a DNA fragment of the RK6 plasmid from a homologue of RK2 traF to a homologue of RK2 traM and all of the remainder of a DNA fragment of the RK6 plasmid from a homologue of RK2 trbB to a homologue of a RK2 trK (preferably when the plasmid is a RK6-type plasmid). For example, with the exception of a gene encoding the essential component, the chromosome comprises all of the remainder of the RK6 plasmid genes from a homologue of RK2 traF to a homologue of RK2 traM and all of the remainder of the RK6 plasmid genes from a homologue of RK2 trbB to a homologue of RK2 trK (preferably when the plasmid is a RK6-type plasmid). For example, the chromosome comprises (in the following order 5' to 3', or 3' to 5') RK6 homologues of the following RK2 tra1 genes: traFGHIXJKLM, or traKLM, or traJXIHGF. For example, the chromosome comprises (in the following order 5' to 3', or 3' to 5') RK6 homologues of the following RK2 tra2 genes: trbBCDEFGHIJKL or trbBCEFGHJL.

For example, with the exception of a gene encoding the essential component, the chromosome comprises all of the remainder of a DNA fragment of the RP4 plasmid from a homologue of RK2 traF to a homologue of RK2 traM and all of the remainder of a DNA fragment of the RP4 plasmid from a homologue of RK2 trbB to a homologue of a RK2 trK (preferably when the plasmid is a RP4-type plasmid). For example, with the exception of a gene encoding the essential component, the chromosome comprises all of the remainder of the RP4 plasmid genes from a homologue of RK2 traF to a homologue of RK2 traM and all of the remainder of the RP4 plasmid genes from a homologue of RK2 trbB to a homologue of RK2 trK (preferably when the plasmid is a RP4-type plasmid). For example, the chromosome comprises (in the following order 5' to 3', or 3' to 5') RP4 homologues of the following RK2 tra1 genes: traFGHIXJKLM, or traKLM, or traJXIHGF. For example, the chromosome comprises (in the following order 5' to 3', or 3' to 5') RP4 homologues of the following RK2 tra2 genes: trbBCDEFGHIJKL or trbBCEFGHJL.

Preferably, the second DNA or a chromosome comprising the second DNA is devoid of a oriT. Preferably, the third DNA or a chromosome comprising the third DNA is devoid of a oriT.

For example, the oriT is only comprised by the first DNA (or plasmid comprising this) and is not comprised by any other DNA in the carrier cell. Thus, transfer is confined just to the first DNA or its plasmid.

The first DNA in all embodiments and configurations may preferably not be comprised by a runaway replication plasmid. Naturally occurring plasmids are present within host cells at a characteristic concentration (referred to herein as a particular plasmid "copy number"). Mutations that destroy the elements of the control cause an over-replication phenotype that manifests itself by an increase in the plasmid copy number ("copy-up" phenotype). In extreme cases of copy-up mutations, plasmid replication is completely unchecked due to the loss of copy control mechanisms. This is referred to as "runaway plasmid replication" or simply "runaway replication" and the plasmid engaging in such runaway replication is a “runaway replication plasmid”.

In an example, the invention relates to a composition comprising a pluralty of carrier cells of the invention (eg, wherein copies of the first DNA are comprised by respective plasmids). Optionally, all of the carrier cells comprise identical said first and second DNAs. Optionally, the plurality comprises a first sub-population of carrier cells (first cells) and a second sub-population of carrier cells (second cells) wherein the first cells comprise indentical first DNAs and the second cells comprise indentical first DNAs (which are different from the first DNAs of the first cells). For example, the former DNAs comprise a NSI that is different from the NSI comprised by the other DNAs. For example, the first DNAs encode a first guide RNA or crRNA and the second DNAs encode a second guide RNA or crRNA, wherein the first guide RNA/crRNA is capable of hybridizing to a first protospacer sequence in first target cells; and the second guide RNA/crRNA is capable of hybridizing to a second protospacer sequence in second target cells, wherein the protospacers are different. Optionally, the first target cells are different from the second target cells. Optionally, the first target cells are of the same species or strain as the second target cells. Alternatively, the first target cells are of species or strain that is different from the species or strain of the second target cells (in this way a cocktail of carrier cells is provided, eg, for administration to a human or animal or plant, to target and kill a plurality of target cells of different species or strains).

In an example, the or each first DNA comprises a plurality (eg, a first and a second) NSIs wherein a first NSI is different from a second NSI (eg, they encode different proteins or RNAs, such as different guide RNAs or crRNAs). In an example, the or each first DNA comprises 2, 3, 4, 5, 6, 7, 8, 9,10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 different types of NSIs. In an example, the or each first DNA comprises NSIs encoding 2, 3, 4, 5, 6, 7, 8, 9,10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 different

guide RNAs. In an example, the or each first DNA comprises NSIs encoding 2, 3, 4, 5, 6, 7, 8, 9,10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 different crRNAs. In an example, the or each first DNA comprises NSIs encoding at least 2, 3, 4, 5, 6, 7, 8, 9,10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 different guide RNAs. In an example, the or each first DNA comprises NSIs encoding at least 2, 3, 4, 5, 6, 7, 8, 9,10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 different crRNAs.

Optionally, the composition is comprised by a liquid (eg, an aqueous liquid or in water), the composition comprising the carrier cells at an amount of from 1 x 10^3 to 1 x 10^{10} (eg, from 1 x 10^4 to 1 x 10^{10}; from 1 x 10^4 to 1 x 10^9; from 1 x 10^4 to 1 x 10^8; from 1 x 10^4 to 1 x 10^7; from 1 x 10^3 to 1 x 10^{10}; from 1 x 10^3 to 1 x 10^9; from 1 x 10^3 to 1 x 10^8; from 1 x 10^3 to 1 x 10^7; from 1 x 10^5 to 1 x 10^{10}; from 1 x 10^5 to 1 x 10^9; from 1 x 10^5 to 1 x 10^8; from 1 x 10^5 to 1 x 10^7; from 1 x 10^6 to 1 x 10^{10}; from 1 x 10^6 to 1 x 10^9; from 1 x 10^6 to 1 x 10^8; or from 1 x 10^6 to 1 x 10^7) cfu/ml. For example, the liquid is a beverage, such for human or animal consumption. For example, the beverage is a livestock beverage, eg, a poultry beverage (ie, a beverage for consumption by poultry, such as chicken).

In an example, the composition is a dietary (eg, dietary supplement) composition for consumption by humans or animals. In an example, the composition is a slimming composition for consumption by humans or animals. In an example, the composition is a growth promotion composition for consumption by humans or animals. In an example, the composition is a body buidling composition for consumption by humans. In an example, the composition is a probiotic composition for consumption by humans or animals. In an example, the composition is a biocidal composition for consumption by humans or animals. In an example, the composition is a pesticidal composition for consumption by humans or animals. In an example, the composition is a zoonosis control composition for consumption by animals.

In an example, the composition comprises vitamins in addition to the carrier cells. In an example, the composition comprises vitamin A, B (eg, B12), C, D, E and/or K in addition to the carrier cells. In an example, the composition comprises lipids in addition to the carrier cells. In an example, the composition comprises carbohydrates in addition to the carrier cells. In an example, the composition comprises proteins and/or amino acids in addition to the carrier cells. In an example, the composition comprises minerals in addition to the carrier cells. In an example, the composition comprises metal ions (eg, Mg^{2+}, Cu^{2+} and/or Zn^{2+}) in addition to the carrier cells. In an example, the composition comprises sodium ions, potassium ions, magnesium ions, calcium ions, manganese ions, iron ions, cobalt ions, copper ions, zinc ions and/or molybdenum ions.

In an example, the composition is a plant fertilizer composition. In an example, the composition is a herbicide. In an example, the composition is a pesticide composition for application to plants.

In any embodiment or example, where appropriate: The plants are, for example, crop plants. The plants are, for example, wheat. The plants are, for example, corn. The plants are, for example, maize. The plants are, for example, fruiting plants. The plants are, for example, vegetable plants. The plants are, for example, tomato plants. The plants are, for example, potato plants. The plants are, for example, grass plants. The plants are, for example, flowering plants. The plants are, for example, trees. The plants are, for example, shrubs.

In an example, the composition is for environmental application, wherein the environment is an outdoors environment (eg, application to a field or waterway or reservoir).

In an example, the composition is comprised by a food or food ingredient (eg, for human or animal consumption). In an example, the composition is comprised by a beverage or beverage ingredient (eg, for human or animal consumption).

In an example the target cell(s) are human biofilm cells, eg, wherein the biofilm is a gut, skin, lung, eye, nose, ear, gastrointestinal tract (GI tract), stomach, hair, kidney, urethra, bronchiole, oral cavity, mouth, liver, heart, anus, rectum, bladder, bowel, intestine, penis, vagina or scrotum biofilm. In an example the target cell(s) are animal biofilm cells, eg, wherein the biofilm is a gut, skin, lung, eye, nose, ear, gastrointestinal tract (GI tract), caecum, jejunum, ileum, colon, stomach, hair, feather, scales, kidney, urethra, bronchiole, oral cavity, mouth, liver, spleen, heart, anus, rectum, bladder, bowel, intestine, penis, vagina or scrotum biofilm. For example, the biofilm is a bird (eg, chicken) caecum biofilm. For example, the biofilm is a bird (eg, chicken) gastrointestinal tract (GI tract), caecum, jejunum, ileum, colon or stomach biofilm.

In an example, any method herein is *ex vivo*. In an example, a method herein is *in vivo*. In an example, a method herein is *in vitro*. In an example, a method herein is carried out in an environment, eg, in a domestic (such as in a house), industrial (such as in a factory) or agricultural environment (such as in a field). In an example, a method herein is carried out in or on a container; or on a surface.

In an example, the NSI (or a RNA product thereof) is capable of recombination with the target cell chromosome or an episome comprised by the target cell to modify the chromosome or episome. Optionally, this is carried out in a method wherein the chromosome or episome is cut (eg, at a

predetermined site using a guided nuclease, such as a Cas, TALEN, zinc finger nuclease or meganuclease) and simultaneously or sequentially the first DNA is introduced into the target cell by conjugation with the carrier cell and the NSI or a sequence thereof is inserted into the chromosome or episome at or adjacent the cut site.

In an example the first DNA comprises one or more components of a CRISPR/Cas system operable to perform protospacer cutting in the target cell (eg, wherein the protospacer comprises 10-20, 10-30, 10-40, 10-100, 12-15 or 12-20 consecutive nucleotides that are capable of hybridizing in the target cell with a crRNA or gRNA encoded by the NSI).

For example, the system is a Type I, II, III, IV or V CRISPR/Cas system.

In an example, the NSI encodes a Cas9 (and optionally a second, different, Cas, such as a Cas3, Cas9, Cpf1, Cas13a, Cas13b or Cas10). In an example, the NSI encodes a Cas3 (and optionally a second, different, Cas, such as a Cas3, Cas9, Cpf1, Cas13a, Cas13b or Cas10). In an example, the NSI encodes a Cas selected from a Cas3, Cas9, Cpf1, Cas13a, Cas13b and Cas10. Additionally or alternatively, the first DNA (eg, the NSI) encodes a guide RNA or crRNA or tracrRNA. For example, the guide RNA or crRNA or tracrRNA is cognate to (ie, operable with in the target cell) the first Cas.

In an example, a Cas herein is a Cas9. In an example, a Cas herein is a Cas3. The Cas may be identical to a Cas encoded by the target bacteria.

In an embodiment, the presence in the target bacterium of the NSI or its encoded protein or RNA mediates target cell killing, or downregulation of growth or propagation of target cells. In an embodiment, the presence in the target bacterium of the NSI or its encoded protein or RNA mediates switching off of expression of one or more RNA or proteins encoded by the target cell genome, or downregulation thereof.

In an embodiment, the presence in the target bacterium of the NSI or its encoded protein or RNA mediates upregulation of growth or propagation of the target cell. In an embodiment, the presence in the target bacterium of the NSI or its encoded protein or RNA mediates switching on of expression of one or more RNA or proteins encoded by the target cell genome, or upregulation thereof.

In an embodiment, the NSI encodes a component of a CRISPR/Cas system that is toxic to the target bacterium.

In an embodiment, the first DNA is comprised by a plasmid or shuttle vector. In an embodiment, the second DNA is comprised by a vector (eg, a plasmid or shuttle vector), helper phage (eg, a helper phagemid) or is integrated in the genome of a host bacterial cell.

Optionally, the target cell is devoid of a functional endogenous CRISPR/Cas system before transfer therein of the first DNA, eg, a first DNA comprising component of an exogenous CRISPR/Cas system that is functional in the target cell and toxic to the target cell. An embodiment provides an antibacterial composition comprising a plurality of carrier cells of the invention, wherein each target cell is optionally according to this paragraph, for administration to a human or animal subject for medical use.

In an example, the composition of the invention is a herbicide, pesticide, insecticide, plant fertilizer or cleaning agent.

Optionally, target bacteria herein are comprised by a microbiome of the subject, eg, a gut microbiome. Altertnatively, the microbiome is a skin, scalp, hair, eye, ear, oral, throat, lung, blood, rectal, anal, vaginal, scrotal, penile, nasal or tongue microbiome.

In an example the subject (eg, human or animal) is further administered a medicament simultaneously or sequentially with the carrier cell administration. In an example, the medicament is an antibiotic, antibody, immune checkpoint inhibitor (eg, an anti-PD-1, anti-PD-L1 or anti-CTLA4 antibody), adoptive cell therapy (eg, CAR-T therapy) or a vaccine.

In an embodiment, the NSI encodes a guided nuclease, such as a Cas nuclease, TALEN, zinc finger nuclease or meganuclease. Thus, the toxic agent may comprise a guided nuclease, such as a Cas nuclease, TALEN, zinc finger nuclease or meganuclease. Optionally, the NSI encodes a restriction nuclease that is capable of cutting the chromosome of the target cell.

Optionally, the composition is a pharmaceutical composition for use in medicine practised on a human or animal subject.

In an example, the animal is a livestock or companion pet animal (eg, a cow, pig, goat, sheep, horse, dog, cat or rabbit). In an example, the animal is an insect (an insect at any stage of its lifecycle, eg, egg, larva or pupa). In an example, the animal is a protozoan. In an example, the animal is a cephalopod.

Optionally, the composition is a herbicide, pesticide, food or beverage processing agent, food or beverage additive, petrochemical or fuel processing agent, water purifying agent, cosmetic additive, detergent additive or environmental (eg, soil) additive or cleaning agent.

The invention also provides: A target bacterial cell or a plurality of target bacterial cells each comprising a said first DNA.

For example the carrier bacteria are *Lactobacillus* (eg, *L reuteri* or *L lactis*), *E coli*, *Bacillus* or *Streptococcus* (eg, *S thermophilus)* bacteria. Usefully, the carrier can provide protection for the first DNA from the surrounding environment. The use of a carrier may be useful for oral administration or other routes where the carrier can provide protection for the first DNA from the acid stomach or other harsh environments in the subject. Furthermore, the carrier can be formulated into a beverage, for example, a probiotic drink, eg, an adapted Yakult (trademark), Actimel (trademark), Kevita (trademark), Activia (trademark), Jarrow (trademark) or similar drink for human consumption.

Optionally, the carrier cell(s) or composition are for administration to a human or animal subject for medical use, comprising killing target bacteria using the agent or expression product of the NSI, wherein the target bacteria mediate as disease or condition in the subject. In an example, when the subject is a human, the subject is not an embryo. In an example, the carrier cells are probiotic in the subject.

The invention also provides: A method of killing target bacterial cells in an environment, optionally wherein the method is not practised on a human or animal body, wherein the method comprises exposing the environment to the carrier cell(s) or composition of the invention and allowing the product of the NSI to be expressed in the target cells, wherein the target bacteria are killed in the presence of said product. For example, the product encodes a CRISPR/Cas system or component thereof, such as a system or component disclosed herein. Thus, the system may be capable of recognisisng and cutting a chromosomal protopspacer sequence of the target cell, whereby the target cell is killed. Optionally, in a further step killed target cells are isolated.

The invention also provides: Use of the composition or cell(s) of the invention, in the manufacture of an antibacterial agent that kills target bacteria, for the treatement of a disease or condition in a human or animal subject comprising the target bacteria.

Optionally, the environment is a microbiome of soil; a plant, part of a part (e.g., a leaf, fruit, vegetable or flower) or plant product (e.g., pulp); water; a waterway; a fluid; a foodstuff or ingredient thereof; a beverage or ingredient thereof; a medical device; a cosmetic; a detergent; blood; a bodily fluid; a medical apparatus; an industrial apparatus; an oil rig; a petrochemical processing, storage or transport apparatus; a vehicle or a container.

Optionally, the environment is an *ex vivo* bodily fluid (e.g., urine, blood, blood product, sweat, tears, sputum or spit), bodily solid (e.g., faeces) or tissue of a human or animal subject that has been administered the composition.

Optionally, the environment is an *in vivo* bodily fluid (e.g., urine, blood, blood product, sweat, tears, sputum or spit), bodily solid (e.g., faeces) or tissue of a human or animal subject that has been administered the composition.

In an embodiment, the first DNA is comprised by a phagemid or cloning vector (eg, a shuttle vector, eg, a pUC vector).

In an embodiment, the second DNA is comprised by the bacterial carrier cell chromosome.

Optionally, the toxic agent comprises one or more components of a CRISPR/Cas system, eg, a DNA sequence encoding one or more components of Type I Cascade (eg, CasA).

Optionally, the toxic agent comprises a DNA sequence encoding guided nuclease, such as a Cas nuclease, TALEN, zinc finger nuclease or meganuclease.

In an example, the carrier cell(s) or composition are comprised by a medical container, eg, a syringe, vial, IV bag, inhaler, eye dropper or nebulizer. In an example, the carrier cell(s) or composition are comprised by a sterile container. In an example, the carrier cell(s) or composition are comprised by a medically-compatible container. In an example, the carrier cell(s) or composition are comprised by a fermentation vessel, eg, a metal, glass or plastic vessel. In an example, the carrier cell(s) or composition are comprised by an agricultural apparatus. In an example, the carrier cell(s) or composition are comprised by food production or processing apparatus. In an example, the carrier cell(s) or composition are comprised by a horticultural apparatus. In an example, the carrier cell(s) or composition are comprised by a farming apparatus. In an example, the carrier cell(s) or composition are comprised by petrochemicals recovery or processing apparatus. In an example, the carrier cell(s)

or composition are comprised by a distillation apparatus. In an example, the carrier cell(s) or composition are comprised by cell culture vessel (eg, having a capacity of at least 50, 100, 1000, 10000 or 100000 litres). Additionally or alternatively, the target cell(s) are comprised by any of these apparatus etc.

In an example, the carrier cell(s) or composition are comprised by a medicament, e,g in combination with instructions or a packaging label with directions to administer the medicament by oral, IV, subcutaneous, intranasal, intraocular, vaginal, topical, rectal or inhaled administration to a human or animal subject. In an example, the carrier cell(s) or composition are comprised by an oral medicament formulation. In an example, the carrier cell(s) or composition are comprised by an intranasal or ocular medicament formulation. In an example, the carrier cell(s) or composition are comprised by a personal hygiene composition (eg, shampoo, soap or deodorant) or cosmetic formulation. In an example, th the carrier cell(s) or composition are comprised by a detergent formulation. In an example, the carrier cell(s) or composition are comprised by a cleaning formulation, eg, for cleaning a medical or industrial device or apparatatus. In an example, the carrier cell(s) or composition are comprised by foodstuff, foodstuff ingredient or foodstuff processing agent. In an example, the carrier cell(s) or composition are comprised by beverage, beverage ingredient or beverage processing agent. In an example, the carrier cell(s) or composition are comprised by a medical bandage, fabric, plaster or swab. In an example, the carrier cell(s) or composition are comprised by a herbicide or pesticide. In an example, the carrier cell(s) or composition are comprised by an insecticide.

In an example, the CRISPR/Cas component(s) are component(s) of a Type I CRISPR/Cas system. In an example, the CRISPR/Cas component(s) are component(s) of a Type II CRISPR/Cas system. In an example, the CRISPR/Cas component(s) are component(s) of a Type III CRISPR/Cas system. In an example, the CRISPR/Cas component(s) are component(s) of a Type IV CRISPR/Cas system. In an example, the CRISPR/Cas component(s) are component(s) of a Type V CRISPR/Cas system. In an example, the CRISPR/Cas component(s) comprise a Cas9-encoding nucleotide sequence (eg, *S pyogenes* Cas9, *S aureus* Cas9 or *S thermophilus* Cas9). In an example, the CRISPR/Cas component(s) comprise a Cas3-encoding nucleotide sequence (eg, *E coli* Cas3, C dificile Cas3 or *Salmonella* Cas3). In an example, the CRISPR/Cas component(s) comprise a Cpf-encoding nucleotide sequence. In an example, the CRISPR/Cas component(s) comprise a CasX-encoding nucleotide sequence. In an example, the CRISPR/Cas component(s) comprise a CasY-encoding nucleotide sequence.

In an example, each carrier cell encodes a CRISPR/Cas component or protein of interest from a nucleotide sequence (NSI) comprising a promoter that is operable in the target bacteria.

Optionally, target bacteria are gram negative bacteria (eg, a spirilla or vibrio). Optionally, target bacteria are gram positive bacteria. Optionally, target bacteria are mycoplasma, chlamydiae, spirochete or mycobacterium bacteria. Optionally, target bacteria are *Streptococcus* (eg, *pyogenes* or *thermophilus*). Optionally, target bacteria are *Staphylococcus* (eg, *aureus*, eg, MRSA). Optionally, target bacteria are *E. coli (eg,* O157: H7), eg, wherein the Cas is encoded by the vecor or an endogenous target cell Cas nuclease (eg, Cas3) activity is de-repressed. Optionally, target bacteria are *Pseudomonas* (eg, *syringae* or *aeruginosa*). Optionally, target bacteria are *Vibro* (eg, *cholerae* (eg, O139) or *vulnificus*). Optionally, target bacteria are *Neisseria* (eg, *gonnorrhoeae* or *meningitidis*). Optionally, target bacteria are *Bordetella* (eg, *pertussis*). Optionally, target bacteria are *Haemophilus* (eg, *influenzae*). Optionally, target bacteria are *Shigella* (eg, *dysenteriae).* Optionally, target bacteria are *Brucella* (eg, *abortus).* Optionally, target bacteria are *Francisella* host. Optionally, target bacteria are *Xanthomonas*. Optionally, target bacteria are *Agrobacterium*. Optionally, target bacteria are *Erwinia*. Optionally, target bacteria are *Legionella* (eg, *pneumophila*). Optionally, target bacteria are *Listeria* (eg, *monocytogenes*). Optionally, target bacteria are *Campylobacter* (eg, *jejuni*). Optionally, target bacteria are *Yersinia* (eg, *pestis*). Optionally, target bacteria are *Borelia* (eg, *burgdorferi*). Optionally, target bacteria are *Helicobacter* (eg, *pylori*). Optionally, target bacteria are *Clostridium* (eg, *dificile* or *botulinum*). Optionally, target bacteria are *Erlichia* (eg, *chaffeensis*). Optionally, target bacteria are *Salmonella* (eg, *typhi* or *enterica*, eg, serotype typhimurium, eg, DT 104). Optionally, target bacteria are *Chlamydia* (eg, *pneumoniae*). Optionally, target bacteria are *Parachlamydia* host. Optionally, target bacteria are *Corynebacterium* (eg, *amycolatum*). Optionally, target bacteria are *Klebsiella* (eg, *pneumoniae*). Optionally, target bacteria are *Enterococcus* (eg, *faecalis* or *faecim,* eg, linezolid-resistant). Optionally, target bacteria are *Acinetobacter* (eg, *baumannii*, eg, multiple drug resistant).

Further examples of target cells are as follows:-

1. Optionally the target bacteria are *Staphylococcus aureus* cells, eg, resistant to an antibiotic selected from methicillin, vancomycin, linezolid, daptomycin, quinupristin, dalfopristin and teicoplanin.

2. Optionally the target bacteria are *Pseudomonas aeuroginosa* cells, eg, resistant to an antibiotic selected from cephalosporins (eg, ceftazidime), carbapenems (eg, imipenem or meropenem), fluoroquinolones, aminoglycosides (eg, gentamicin or tobramycin) and colistin.

3. Optionally the target bacteria are *Klebsiella* (eg, *pneumoniae*) cells, eg, resistant to carbapenem.

4. Optionally the target bacteria are *Streptoccocus (eg, thermophilus*, *pneumoniae* or pyogenes) cells, eg, resistant to an antibiotic selected from erythromycin, clindamycin, beta-lactam, macrolide, amoxicillin, azithromycin and penicillin.

5. Optionally the target bacteria are *Salmonella* (eg, serotype Typhi) cells, eg, resistant to an antibiotic selected from ceftriaxone, azithromycin and ciprofloxacin.

6. Optionally the target bacteria are *Shigella* cells, eg, resistant to an antibiotic selected from ciprofloxacin and azithromycin.

7. Optionally the target bacteria are *Mycobacterium tuberculosis* cells, eg, resistant to an antibiotic selected from Resistance to isoniazid (INH), rifampicin (RMP), fluoroquinolone, amikacin, kanamycin and capreomycin and azithromycin.

8. Optionally the target bacteria are *Enterococcus* cells, eg, resistant to vancomycin.

9. Optionally the target bacteria are *Enterobacteriaceae* cells, eg, resistant to an antibiotic selected from a cephalosporin and carbapenem.

10. Optionally the target bacteria are *E. coli* cells, eg, resistant to an antibiotic selected from trimethoprim, itrofurantoin, cefalexin and amoxicillin.

11. Optionally the target bacteria are *Clostridium* (eg, *dificile*) cells, eg, resistant to an antibiotic selected from fluoroquinolone antibiotic and carbapenem.

12. Optionally the target bacteria are *Neisseria gonnorrhoea* cells, eg, resistant to an antibiotic selected from cefixime (eg, an oral cephalosporin), ceftriaxone (an injectable cephalosporin), azithromycin and tetracycline.

13. Optionally the target bacteria are *Acinetoebacter baumannii* cells, eg, resistant to an antibiotic selected from beta-lactam, meropenem and a carbapenem.

14. Optionally the target bacteria are *Campylobacter* (eg, *jejuni*) cells, eg, resistant to an antibiotic selected from ciprofloxacin and azithromycin.

15. Optionally, the target cell(s) produce Beta (β)-lactamase (eg, ESBL-producing *E. coli* or ESBL-producing *Klebsiella*).

16. Optionally, the target cell(s) are bacterial cells that are resistant to an antibiotic recited in any one of examples 1 to 14.

In an example, the target cell(s) is a cell of a species selected from *Shigella, E coli, Salmonella, Serratia, Klebsiella, Yersinia, Pseudomonas* and *Enterobacter.*

Optionally, the composition comprises carrier cells that are each or in combination capable of conjugative transfer of first DNAs into target cells of species selected from two or more of *Shigella, E coli, Salmonella, Serratia, Klebsiella, Yersinia, Pseudomonas* and *Enterobacter*.

In an example, the reduction in growth or proliferation of carrier cells is at least 50, 60, 70, 80, 90 or 95%. Optionally, the composition or carrier cell(s) are administered simultaneously or sequentially with an an antibiotic that is toxic to the target cells. For example, the antibiotic can be any antibiotic disclosed herein.

Optioanlly, the expression of the NSI is under the control of an inducible promoter that is operable in the target cell. Optioanlly, the expression of the NSI is under the control of a constitutive promoterthat is operable in the target cell.

In embodiments, the first DNA (eg, comprised by a plasmid) contains a screenable or selectable marker gene. For example, the selectable marker gene is an antibiotic resistance gene.

The carrier bacteria can be bacteria of a species or genus selected from those appearing in Table 5. For example, the species is found in warm-blooded animals (eg, livestock vertebrates). For example, the species is found in humans. For example, the species is found in plants. Preferably, non-pathogenic bacteria that colonize the non-sterile parts of the human or animal body (e.g., skin, digestive tract, urogenital region, mouth, nasal passages, throat and upper airway, ears and eyes) are utilized as carrier cells, and in an example the methodology of the invention is used to combat a target cell bacterial infection of such a part of the body of a human or animal. In another embodiment, the infection is systemic infection. Examples of particularly preferred carrier bacterial species include, but are not limited to: non-pathogenic strains of *Escherichia coli (E. coli* F18, S17 and *E. coli strain* Nissle), various species of *Lactobacillus* (such as *L. casei, L. plantarum, L. paracasei, L. acidophilus, L. fermentum, L. zeae* and *L. gasseri)*, or other nonpathogenic or probiotic skin- or GI colonizing bacteria such as *Lactococcus, Bifidobacteria, Eubacteria*, and bacterial mini-cells, which are anucleoid cells destined to die but still capable of transferring plasmids (see; e.g., Adler et al., Proc. Natl. Acad. Sci. USA 57; 321-326, 1970; Frazer and Curtiss III, Current Topics in Microbiology and Immunology 69: 1-84, 1975; U.S. Patent No. 4,968,619 to Curtiss III). In some embodiments, the target recipient cells are pathogenic bacteria comprised by a human, animal or plant, eg, on the skin or in the digestive tract, urogenital region, mouth, nasal passage, throat and upper airway, eye(s) and ear(s). Of particular interest for targeting and eradication are pathogenic strains of *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus pneumoniae* and other species,

Enterobacter spp., Enterococcus spp. and Mycobacterium tuberculosis. In an example, the target cell genus or species is any genus or species listed in Table 5.

The present invention finds use with a wide array of settings or environments, eg, in therapeutic, agricultural, or other settings, including, but not limited to, those described in U.S. patents 6,271,359, 6,261,842, 6,221,582, 6,153,381, 6,106,854, and 5,627,275. Others are also discussed herein, and still others will be readily apparent to those of skill in the art.

Numerous types of plasmids comprising the first DNA are suitable for use in the present invention. In view of this, one of skill in the art will appreciate that a single carrier bacterial strain might harbor more than one type of such plasmid (eg, differing in the antibacterial agent that they encode). Further, in another example two or more different carrier bacterial strains, each containing one or more such plasmids, may be combined for a multi-target effect, ie, for killing two or more different target species or strains, or for killing the cells of the same species or strain of target cell.

The present invention finds utility for treatment of humans and in a variety of veterinary, agronomic, horticultural and food processing applications. For human and veterinary use, and depending on the cell population or tissue targeted for protection, the following modes of administration of the carrier bacteria of the invention are contemplated: topical, oral, nasal, ocular, aural, pulmonary (e.g., via an inhaler), ophthalmic, rectal, urogenital, subcutaneous, intraperitoneal and intravenous. The bacteria may be supplied as a pharmaceutical composition, in a delivery vehicle suitable for the mode of administration selected for the patient being treated. The term "patient" or "subject" as used here refers to humans or animals (animals being particularly useful as models for clinical efficacy of a particular donor strain, for example, or being farmed or livestock animals). Commercially-relevant animals are chicken, turkey, duck, catfish, salmon, cod, herring, lobster, shrimp, prawns, cows, sheep, goats, pigs, goats, geese or rabbits.

For example, to deliver the carrier bacteria to the gastrointestinal tract or to the nasal passages, the preferred mode of administration may be by oral ingestion or nasal aerosol, or by feeding (alone or incorporated into the subject's feed or food and/or beverage, such as drinking water). In this regard, the carrier cells may be comprised by a food of livestock (or farmed or companion animal), eg, the carrier bacteria are comprised by a feed additive for livestock. Alternatively, the additive is a beverage (eg, water) additive for livestock. It should be noted that probiotic bacteria, such as *Lactobacillus acidophilus*, are sold as gel capsules containing a lyophilized mixture of bacterial cells and a solid support such as mannitol. When the gel capsule is ingested with liquid, the lyophilized

cells are re-hydrated and become viable, colonogenic bacteria. Thus, in a similar fashion, carrier bacterial cells of the present invention can be supplied as a powdered, lyophilized preparation in a gel capsule, or in bulk, eg, for sprinkling onto food or beverages. The re-hydrated, viable bacterial cells will then populate and/or colomze sites throughout the upper and/or lower gastrointestinal system, and thereafter come into contact with the target bacteria.

For topical applications, the carrier bacteria may be formulated as an ointment or cream to be spread on the affected skin surface. Ointment or cream formulations are also suitable for rectal or vaginal delivery, along with other standard formulations, such as suppositories. The appropriate formulations for topical, vaginal or rectal administration are well known to medicinal chemists.
The present invention will be of particular utility for topical or mucosal administrations to treat a variety of bacterial infections or bacterially related undesirable conditions. Some representative examples of these uses include treatment of (1) conjunctivitis, caused by *Haemophilus sp.*, and corneal ulcers, caused by *Pseudomonas aeruginosa*; (2) otititis externa, caused by *Pseudomonas aeruginosa*; (3) chronic sinusitis, caused by many Gram-positive cocci and Gram-negative rods, or for general decontamination of bronchii; (4) cystic fibrosis, associated with *Pseudomonas aeruginosa*; (5) enteritis, caused by *Helicobacter pylori* (eg, to treat or prevent gastric ulcers), *Escherichia coli*, *Salmonella typhimurium, Campylobacter* or *Shigella* sp. ; (6) open wounds, such as surgical or non-surgical, eg, as a prophylactic measure; (7) burns to eliminate *Pseudomonas aeruginosa* or other Gram-negative pathogens; (8) acne, eg, caused by *Propionobacter acnes*; (9) nose or skin infection, eg, caused by metlncillin resistant *Staphylococcus aureus* (MSRA); (10) body odor, eg, caused by Gram-positive anaerobic bacteria (i.e., use of carrier cells in deodorants); (11) bacterial vaginosis, eg, associated with *Gardnerella vaginalis* or other anaerobes; and (12) gingivitis and/or tooth decay caused by various organisms.

In one example, the target cells are *E coli* cells and the disease or condition to be treated in a human is a uterine tract infection or a ventilator associated infection, eg, pneumonia.

In other embodiments, the carrier cells of the present invention find application in the treatment of surfaces for the removal or attenuation of unwanted target bacteria, for example use in a method of treating such a surface or an environment comprising target bacteria, wherein the method comprises contacting the surface or environment with carrier bacteria of the invention, allowing conjugative transfer of the first DNA of the invention from the carrier to the target bacteria, and allowing the antibacterial agent to kill target cells. For example, surfaces that may be used in invasive treatments such as surgery, catheterization and the like may be treated to prevent infection of a subject by

bacterial contaminants on the surface. It is contemplated that the methods and compositions of the present invention may be used to treat numerous surfaces, objects, materials and the like (e.g., medical or first aid equipment, nursery and kitchen equipment and surfaces) to control bacterial contamination thereon.

Pharmaceutical preparations or other compositions comprising the carrier bacteria may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to a physically discrete unit of the pharmaceutical preparation appropriate for the patient or plant or environment or surface undergoing treatment. Each dosage should contain a quantity of the carrier bacteria calculated to produce the desired antibacterial effect in association with the selected carrier. Procedures for determining the appropriate dosage unit are well known to those skilled in the art. Dosage units may be proportionately increased or decreased based on the weight of a patient, plant, surface or environment. Appropriate concentrations for achieving eradication of pathogenic target cells (eg, comprised by a tissue of the patient) may be determined by dosage concentration curve calculations, as known in the art.

Other uses for the carrier bacteria of the invention are also contemplated. These include a variety agricultural, horticultural, environmental and food processing applications. For example, in agriculture and horticulture, various plant pathogenic bacteria may be targeted in order to minimize plant disease. One example of a plant pathogen suitable for targeting is *Erwinia* (eg, *E amylovora*, the causal agent of fire blight). Similar strategies may be utilized to reduce or prevent wilting of cut flowers. For veterinary or animal farming, the carrier cells of the invention may be incorporated into animal feed (chicken, swine, poultry, goat, sheep, fish, shellfish or cattle feed) to reduce bio-burden or to eliminate certain pathogenic organisms (e.g., *Salmonella*, such as in chicken, turkey or other poultry). In other embodiments, the invention may be applied on meat or other foods to eliminate unwanted or pathogenic bacteria (e.g., *E. coli* O157:H7 on meat, or *Proteus* spp., one cause of "fishy odour" on seafood).

Environmental utilities comprise, for example, engineering carrier bacteria, eg, *Bacillus thurengiensis* and one of its conjugative plasmids, to deliver and conditionally express an insecticidal agent in addition to or instead of an antibacterial agent (e.g., for the control of mosquitos that disseminate malaria or West Nile virus). In such applications, as well as in the agricultural and horticultural or other applications described above, formulation of the carrier bacteria as solutions, aerosols, or gel capsules are contemplated.

In preferred embodiments of the present invention, certain features are employed in the DNA, plasmids and carrier cells of the invention to minimize potential risks associated with the use of engineered DNA or genetically modified organisms in the environment. For instance, eg, in environmentally sensitive circumstances, it may be advantageous to utilise non-self-transmissible DNA or plasmids. Instead, the DNA or plasmids will be mobilisable by conjugative machinery but will not be self-transmissible. This may be accomplished in some embodiments by integrating into the carrier cell chromosome all or some of the tra genes whose products are necessary for the assembly of conjugative machinery. In such embodiments, DNA or plasmids of the invention are configured to possess an origin of transfer (oriT) but not the tra genes that are provided on the cell's chromosome. This feature prevents the recipient killer cell, before or even after it dies, from transferring the killer DNA or plasmid further. Another biosafety feature comprises utilizing conjugation systems with predetermined host-ranges. Certain elements are known to function only in few related bacteria (narrow-host-range) and others are known to function in many unrelated bacteria (broad-host-range or promiscuous) (del Solar et al., Mol. Microbiol. 32: 661-666, 1996; Zatyka and Thomas, FEMS Microbiol. Rev. 21: 291-319, 1998). Also, many of those conjugation systems can function in either gram-positive or gram-negative bacteria but generally not in both (del Solar, 1996, supra; Zatyka and Thomas, 1998).

Inadvertant proliferation of antibiotic resistance may be minimized in embodiments by avoiding the use of antibiotic resistance markers on the DNA or plasmids of the invention which are conjugatively transferred into target cells. In an alternative approach, the gene responsible for the synthesis of an amino acid (i.e. serine) can be mutated, generating the requirement for this amino acid in the donor. Such mutant bacteria will prosper on media lacking serine provided that they contain a plasmid with the ser gene whose product is needed for growth. Thus, the invention contemplates the advantageous use of plasmids containing the ser gene or another nutritional genetic marker. These markers will permit selection and maintenance of the DNA or plasmids in carrier cells. Another biosafety approach comprises the use of restriction-modification systems to modulate the host range of the DNA or plasmids. Conjugation and plasmid establishment are expected to occur more frequently between taxonomically related species in which plasmid can evade restriction systems and replicate. Type II restriction endonucleases make a double-strand break within or near a specific recognition sequence of duplex DNA. Cognate modification enzymes can methylate the same sequence and protect it from cleavage. Restriction-modification systems (RM) are ubiquitous in bacteria and archaebacteria but are absent in eukaryotes. Some of RM systems are plasmid-encoded, while others are on the bacterial chromosome (Roberts and Macelis, Nucl. Acids Res. 24: 223-235, 1998). Restriction enzymes cleave foreign DNA such as viral or plasmid DNA when this DNA has not been

modified by the appropriate modification enzyme. In this way, cells are protected from invasion of foreign DNA. Thus, by using a carrier strain producing one or more methylases, cleavage by one or more restriction enzymes could be evaded. Site-directed mutagenesis is used to produce plasmid DNA that is either devoid of specific restriction sites or that comprises new sites, protecting or making plasmid DNA vulnerable, respectively against endonucleases. Broad-host range plasmids (eg. RP4) may evade restriction systems simply by not having many of the restriction cleavage sites that are typically present on narrow-host plasmids (Willkins et al., 1996, J. Mol. Biol 258, 447-456). Preferred embodiments of the present invention also utilize environmentally safe bacteria as carriers. For example, delivery of DNA vaccines by attenuated intracellular gram-positive and gram-negative bacteria has been reported (Dietrich et al., 2001 Vaccine 19, 2506-2512; Grillot-Courvalin et al, 1999 Current Opinion in Biotech. 10, 477-481). In addition, the donor strain can be one of thousands of harmless bacteria that colonize the non-sterile parts of the body (e.g., skin, gastrointestinal, urogenital, mouth, nasal passages, throat and upper airway systems). Examples of preferred donor (ie, carrier) bacterial species are set forth hereinabove.

In another strategy, non-dividing, non-growing carrier cells are utilized instead of living cells. Minicells and maxicells are well studied model systems of metabolically active but nonviable bacterial cells. Minicells lack chromosomal DNA and are generated by special mutant cells that undergo cell division without DNA replication. If the cell contains a multicopy plasmid, many of the minicells will contain plasmids. Minicells neither divide nor grow. However, minicells that possess conjugative plasmids are capable of conjugal replication and transfer of plasmid DNA to living recipient cells. (Adler et al., 1970, supra; Frazer and Curtiss, 1975, supra; U.S. Patent No. 4,968,619, supra). Maxicells can be obtained from a strain of *E. coli* that carries mutations in the key DNA repair pathways (recA, uvrA and phr). Because maxicells lack so many DNA repair functions, they die upon exposure to low doses of UV. Importantly, plasmid molecules (e.g., pBR322) that do not receive an UV hit continue to replicate. Transcription and translation (plasmid-directed) can occur efficiently under such conditions (Sancar et al., J. Bacteriol. 137: 692-693, 1979), and the proteins made prior to irradiation should be sufficient to sustain conjugation. This is supported by the following two observations: i) that streptomycin-killed cells remain active donors, and ii) that transfer of conjugative plasmids can occur in the presence of antibiotics that prevent de novo gene expression (Heineman and Ankenbauer, 1993, J. Bacteriol. 175, 583-588;Cooper and Heineman, 2000. Plasmid 43, 171-175). Accordingly, UV-treated maxicells will be able to transfer plasmid DNA to live recipients. It should also be noted that the conservation of recA and uvrA genes among bacteria should allow maxicells of donor strains other than *E. coli* to be obtained.

Also contemplated for use in the invention are any of the modified bacteria that cannot function because they contain temperature-sensitive mutation(s) in genes that encode for essential cellular functions (e.g., cell wall, protein synthesis, RNA synthesis, as described, for example, in US patent 4,968,619, supra).

In an alternative, archaea are used instead of bacteria for the carrier cells. Additionally or alternatively, the target cells are archaeal cells.

As used herein, the term "carrier cell" includes dividing and/or non-dividing bacterial cells (minicells and maxicells), or conditionally non-functional cells.

In an example the first DNA is comprised by an engineered RK2 plasmid (ie, a RK2 plasmid that has been modified by recombinant DNA technology or a progeny of such a modified plasmid). Plasmid RK2 is a promiscuous plasmid that can replicate in 29 (and probably many more) gram-negative species (Guiney and Lanka, 1989, p 27-54. In C. M. Thomas (ed) Promiscous plasmids in gram-negative bacteria. London, Ltd London United Kingdom.). Plasmid RK2 is a 60-kb self-transmissible plasmid with a complete nucleotide sequence known (Pansegrau et al., 1994, J. Mol. Biol. 239, 623-663). A minimal replicon derived from this large plasmid has been obtained that is devoid of all its genes except for a trfA gene, that encodes plasmid' s Rep protein called TrfA, and an origin of vegetative replication oriV For a review of RK2 replication and its control by TrfA protein, see Helinski et al., 1996 (In Escherichia coli and Salmonella Cellular and Molecular Biology, Vol. 2 (ed. F. Neidhardt, et al., 2295-2324, ASM Press, Washington D.C.).

In an example the first DNA is comprised by an engineered R6K plasmid (ie, a R6K plasmid that has been modified by recombinant DNA technology or a progeny of such a modified plasmid).

The present invention is optionally for an industrial or domestic use, or is used in a method for such use. For example, it is for or used in agriculture, oil or petroleum industry, food or drink industry, clothing industry, packaging industry, electronics industry, computer industry, environmental industry, chemical industry, aeorspace industry, automotive industry, biotechnology industry, medical industry, healthcare industry, dentistry industry, energy industry, consumer products industry, pharmaceutical industry, mining industry, cleaning industry, forestry industry, fishing industry, leisure industry, recycling industry, cosmetics industry, plastics industry, pulp or paper industry, textile industry, clothing industry, leather or suede or animal hide industry, tobacco industry or steel industry.

The present invention is optionally for use in an industry or the environment is an industrial environment, wherein the industry is an industry of a field selected from the group consisting of the medical and healthcare; pharmaceutical; human food; animal food; plant fertilizers; beverage; dairy; meat processing; agriculture; livestock farming; poultry farming; fish and shellfish farming; veterinary; oil; gas; petrochemical; water treatment; sewage treatment; packaging; electronics and computer; personal healthcare and toiletries; cosmetics; dental; non-medical dental; ophthalmic; non-medical ophthalmic; mineral mining and processing; metals mining and processing; quarrying; aviation; automotive; rail; shipping; space; environmental; soil treatment; pulp and paper; clothing manufacture; dyes; printing; adhesives; air treatment; solvents; biodefence; vitamin supplements; cold storage; fibre retting and production; biotechnology; chemical; industrial cleaning products; domestic cleaning products; soaps and detergents; consumer products; forestry; fishing; leisure; recycling; plastics; hide, leather and suede; waste management; funeral and undertaking; fuel; building; energy; steel; and tobacco industry fields.

In an example, the first DNA comprises a CRISPR array that targets target bacteria, wherein the array comprises one, or two or more different spacers (eg, 2, 3, 4, 5, 6, 7, 8, 9 ,10, 20, 30, 40, 50 or more spacers) for targeting the genome of target bacteria.

In an example, the target bacteria are comprised by an environment as follows. In an example, the environment is a microbiome of a human, eg, the oral cavity microbiome or gut microbiome or the bloodstream. In an example, the environment is not an environment in or on a human. In an example, the environment is not an environment in or on a non-human animal. In an embodiment, the environment is an air environment. In an embodiment, the environment is an agricultural environment. In an embodiment, the environment is an oil or petroleum recovery environment, eg, an oil or petroleum field or well. In an example, the environment is an environment in or on a foodstuff or beverage for human or non-human animal consumption. In an example, the environment is a maritimeenvironment, eg, in seawater or on a boat (eg, in ship or boat ballast water).

In an example, the environment is a a human or animal microbiome (eg, gut, vaginal, scalp, armpit, skin or oral cavity microbiome). In an example, the target bacteria are comprised by a human or animal microbiome (eg, gut, vaginal, scalp, armpit, skin or oral cavity microbiome).

In an example, the carrier bacteria or composition of the invention are administered intranasally, topically or orally to a human or non-human animal, or is for such administration. The skilled person aiming to treat a microbiome of the human or animal will be able to determine the best route of

administration, depending upon the microbiome of interest. For example, when the microbiome is a gut microbiome, administration can be intranasally or orally. When the microbiome is a scalp or armpit microbiome, administration can be topically. When the microbiome is in the mouth or throat, the administration can be orally.

In an example, the environment is harboured by a beverage or water (eg, a waterway or drinking water for human consumption) or soil. The water is optionally in a heating, cooling or industrial system, or in a drinking water storage container.

In an example, the carrier and/or target bacteraia are *Firmicutes* selected from *Anaerotruncus, Acetanaerobacterium, Acetitomaculum, Acetivibrio, Anaerococcus, Anaerofilum, Anaerosinus, Anaerostipes, Anaerovorax, Butyrivibrio, Clostridium, Capracoccus, Dehalobacter, Dialister, Dorea, Enterococcus, Ethanoligenens, Faecalibacterium, Fusobacterium, Gracilibacter, Guggenheimella, Hespellia, Lachnobacterium, Lachnospira, Lactobacillus, Leuconostoc, Megamonas, Moryella, Mitsuokella, Oribacterium, Oxobacter, Papillibacter, Proprionispira,Pseudobutyrivibrio, Pseudoramibacter, Roseburia, Ruminococcus, Sarcina, Seinonella, Shuttleworthia, Sporobacter, Sporobacterium, Streptococcus, Subdoligranulum, Syntrophococcus, Thermobacillus, Turibacter* and *Weisella.*

In an example, the carrier bacteria, composition, use or method is for reducing pathogenic infections or for re-balancing gut or oral biofilm eg, for treating or preventing obesity or disease in a human or animal; or for treating or preventing a GI condition (such as Crohn's disease, IBD or colitis). For example, the DNA, carrier bacteria, composition, use or method is for knocking-down *Salmomnella, Campylobacter, Erwinia, Xanthomonous, Edwardsiella, Pseudomonas, Klebsiella, Pectobacterium, Clostridium dificile* or *E coli* bacteria in a gut biofilm of a human or animal or a plant, preferably in a human or animal.

In an example, the animal is a chicken, eg, and the target bacteria are *Salmomnella* or *Campylobacter*. In an example, the animal is a fish (eg, catfish or salmon) or shellfish (eg, prawn or lobster), eg, and the target bacteria are *Edwardsiella.* In an example, the plant is a potato plant and, eg, the target bacteria are *Pectobacterium*. In an example, the plant is a cabbage plant and, eg, the target bacteria are *Xanthomonous* (eg, *X campestris*). In an example, the plant is a marijuana plant and, eg, the targt bacteria are *Pseudomonas* (eg, *P cannabina* or *P amygdali*), *Agrobacterium* (eg, *A tumefaciens*) or Xanthomonas (eg, *X campestris*). In an example, the plant is a hemp plant and, eg, the targt bacteria

are are *Pseudomonas* (eg, *P cannabina* or *P amygdali*), *Agrobacterium* (eg, *A tumefaciens*) or Xanthomonas (eg, *X campestris*).

In an example, the disease or condition is a cancer, inflammatory or autoimmune disease or condition, eg, obesity, diabetes IBD, a GI tract condition or an oral cavity condition.

Optionally, the environment is comprised by, or the target bacteria are comprised by, a gut biofilm, skin biofilm, oral cavity biofilm, throat biofilm, hair biofilm, armpit biofilm, vaginal biofilm, rectal biofilm, anal biofilm, ocular biofilm, nasal biofilm, tongue biofilm, lung biofilm, liver biofilm, kidney biofilm, genital biofilm, penile biofilm, scrotal biofilm, mammary gland biofilm, ear biofilm, urethra biofilm, labial biofilm, organ biofilm or dental biofilm. Optionally, the environment is comprised by, or the target bacteria are comprised by, a plant (eg, a tobacco, crop plant, fruit plant, vegetable plant or tobacco, eg on the surface of a plant or contained in a plant) or by an environment (eg, soil or water or a waterway or acqueous liquid).

In an example, the carrier cell(s) or composition is for treating a disease or condition in an animal or human, wherein the disease or condition. In an example, the disease or condition is caused by or mediated by an infection of target cells comprised by the subject or patient. In an example, the disease or condition is associated with an infection of target cells comprised by the subject or patient. In an example, a symptom of the disease or condition is an infection of target cells comprised by the subject or patient.

Optionally, the disease or condition of a human or animal subject is selected from

(a) A neurodegenerative disease or condition;
(b) A brain disease or condition;
(c) A CNS disease or condition;
(d) Memory loss or impairment;
(e) A heart or cardiovascular disease or condition, eg, heart attack, stroke or atrial fibrillation;
(f) A liver disease or condition;
(g) A kidney disease or condition, eg, chronic kidney disease (CKD);
(h) A pancreas disease or condition;
(i) A lung disease or condition, eg, cystic fibrosis or COPD;
(j) A gastrointestinal disease or condition;
(k) A throat or oral cavity disease or condition;

(l) An ocular disease or condition;

(m) A genital disease or condition, eg, a vaginal, labial, penile or scrotal disease or condition;

(n) A sexually-transmissible disease or condition, eg, gonorrhea, HIV infection, syphilis or Chlamydia infection;

(o) An ear disease or condition;

(p) A skin disease or condition;

(q) A heart disease or condition;

(r) A nasal disease or condition

(s) A haematological disease or condition, eg, anaemia, eg, anaemia of chronic disease or cancer;

(t) A viral infection;

(u) A pathogenic bacterial infection;

(v) A cancer;

(w) An autoimmune disease or condition, eg, SLE;

(x) An inflammatory disease or condition, eg, rheumatoid arthritis, psoriasis, eczema, asthma, ulcerative colitis, colitis, Crohn's disease or IBD;

(y) Autism;

(z) ADHD;

(aa) Bipolar disorder;

(bb) ALS [Amyotrophic Lateral Sclerosis];

(cc) Osteoarthritis;

(dd) A congenital or development defect or condition;

(ee) Miscarriage;

(ff) A blood clotting condition;

(gg) Bronchitis;

(hh) Dry or wet AMD;

(ii) Neovascularisation (eg, of a tumour or in the eye);

(jj) Common cold;

(kk) Epilepsy;

(ll) Fibrosis, eg, liver or lung fibrosis;

(mm) A fungal disease or condition, eg, thrush;

(nn) A metabolic disease or condition, eg, obesity, anorexia, diabetes, Type I or Type II diabetes.

(oo) Ulcer(s), eg, gastric ulceration or skin ulceration;

(pp) Dry skin;
(qq) Sjogren's syndrome;
(rr) Cytokine storm;
(ss) Deafness, hearing loss or impairment;
(tt) Slow or fast metabolism (ie, slower or faster than average for the weight, sex and age of the subject);
(uu) Conception disorder, eg, infertility or low fertility;
(vv) Jaundice;
(ww) Skin rash;
(xx) Kawasaki Disease;
(yy) Lyme Disease;
(zz) An allergy, eg, a nut, grass, pollen, dust mite, cat or dog fur or dander allergy;
(aaa) Malaria, typhoid fever, tuberculosis or cholera;
(bbb) Depression;
(ccc) Mental retardation;
(ddd) Microcephaly;
(eee) Malnutrition;
(fff) Conjunctivitis;
(ggg) Pneumonia;
(hhh) Pulmonary embolism;
(iii) Pulmonary hypertension;
(jjj) A bone disorder;
(kkk) Sepsis or septic shock;
(lll) Sinusitus;
(mmm) Stress (eg, occupational stress);
(nnn) Thalassaemia, anaemia, von Willebrand Disease, or haemophilia;
(ooo) Shingles or cold sore;
(ppp) Menstruation;
(qqq) Low sperm count.

NEURODEGENERATIVE OR CNS DISEASES OR CONDITIONS FOR TREATMENT OR PREVENTION BY THE INVENTION

In an example, the neurodegenerative or CNS disease or condition is selected from the group consisting of Alzheimer disease , geriopsychosis, Down syndrome, Parkinson's disease, Creutzfeldt-jakob disease, diabetic neuropathy, Parkinson syndrome, Huntington's disease, Machado-Joseph

disease, amyotrophic lateral sclerosis, diabetic neuropathy, and Creutzfeldt Creutzfeldt- Jakob disease. For example, the disease is Alzheimer disease. For example, the disease is Parkinson syndrome.

In an example, wherein the method of the invention is practised on a human or animal subject for treating a CNS or neurodegenerative disease or condition, the method causes downregulation of Treg cells in the subject, thereby promoting entry of systemic monocyte-derived macrophages and/or Treg cells across the choroid plexus into the brain of the subject, whereby the disease or condition (eg, Alzheimer's disease) is treated, prevented or progression thereof is reduced. In an embodiment the method causes an increase of IFN-gamma in the CNS system (eg, in the brain and/or CSF) of the subject. In an example, the method restores nerve fibre and//or reduces the progression of nerve fibre damage. In an example, the method restores nerve myelin and//or reduces the progression of nerve myelin damage. In an example, the method of the invention treats or prevents a disease or condition disclosed in WO2015136541 and/or the method can be used with any method disclosed in WO2015136541 (the disclosure of this document is incorporated by reference herein in its entirety, eg, for providing disclosure of such methods, diseases, conditions and potential therapeutic agents that can be administered to the subject for effecting treatement and/or prevention of CNS and neurodegenerative diseases and conditions, eg, agents such as immune checkpoint inhibitors, eg, anti-PD-1, anti-PD-L1, anti-TIM3 or other antibodies disclosed therein).

CANCERS FOR TREATMENT OR PREVENTION BY THE METHOD

Cancers that may be treated include tumours that are not vascularized, or not substantially vascularized, as well as vascularized tumours. The cancers may comprise non-solid tumours (such as haematological tumours, for example, leukaemias and lymphomas) or may comprise solid tumours. Types of cancers to be treated with the invention include, but are not limited to, carcinoma, blastoma, and sarcoma, and certain leukaemia or lymphoid malignancies, benign and malignant tumours, and malignancies e.g., sarcomas, carcinomas, and melanomas. Adult tumours/cancers and paediatric tumours/cancers are also included.

Haematologic cancers are cancers of the blood or bone marrow. Examples of haematological (or haematogenous) cancers include leukaemias, including acute leukaemias (such as acute lymphocytic leukaemia, acute myelocytic leukaemia, acute myelogenous leukaemia and myeloblasts, promyeiocytic, myelomonocytic, monocytic and erythroleukaemia), chronic leukaemias (such as chronic myelocytic (granulocytic) leukaemia, chronic myelogenous leukaemia, and chronic lymphocytic leukaemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma

(indolent and high grade forms), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, myeiodysplastic syndrome, hairy cell leukaemia and myelodysplasia.

Solid tumours are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumours can be benign or malignant. Different types of solid tumours are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumours, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumour, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous eel! carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumour, cervical cancer, testicular tumour, seminoma, bladder carcinoma, melanoma, and CNS tumours (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medu!loblastoma, Schwannoma craniopharyogioma, ependymoma, pineaioma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, neuroblastoma, retinoblastoma and brain metastases).

AUTOIMMUNE DISEASES FOR TREATMENT OR PREVENTION BY THE METHOD

1. Acute Disseminated Encephalomyelitis (ADEM)
2. Acute necrotizing hemorrhagic leukoencephalitis
3. Addison's disease
4. Agammaglobulinemia
5. Alopecia areata
6. Amyloidosis
7. Ankylosing spondylitis
8. Anti-GBM/Anti-TBM nephritis
9. Antiphospholipid syndrome (APS)
10. Autoimmune angioedema
11. Autoimmune aplastic anemia
12. Autoimmune dysautonomia
13. Autoimmune hepatitis
14. Autoimmune hyperlipidemia

15. Autoimmune immunodeficiency
16. Autoimmune inner ear disease (AIED)
17. Autoimmune myocarditis
18. Autoimmune oophoritis
19. Autoimmune pancreatitis
20. Autoimmune retinopathy
21. Autoimmune thrombocytopenic purpura (ATP)
22. Autoimmune thyroid disease
23. Autoimmune urticaria
24. Axonal & neuronal neuropathies
25. Balo disease
26. Behcet's disease
27. Bullous pemphigoid
28. Cardiomyopathy
29. Castleman disease
30. Celiac disease
31. Chagas disease
32. Chronic fatigue syndrome
33. Chronic inflammatory demyelinating polyneuropathy (CIDP)
34. Chronic recurrent multifocal ostomyelitis (CRMO)
35. Churg-Strauss syndrome
36. Cicatricial pemphigoid/benign mucosal pemphigoid
37. Crohn's disease
38. Cogans syndrome
39. Cold agglutinin disease
40. Congenital heart block
41. Coxsackie myocarditis
42. CREST disease
43. Essential mixed cryoglobulinemia
44. Demyelinating neuropathies
45. Dermatitis herpetiformis
46. Dermatomyositis
47. Devic's disease (neuromyelitis optica)
48. Discoid lupus
49. Dressler's syndrome

50. Endometriosis
51. Eosinophilic esophagitis
52. Eosinophilic fasciitis
53. Erythema nodosum
54. Experimental allergic encephalomyelitis
55. Evans syndrome
56. Fibromyalgia
57. Fibrosing alveolitis
58. Giant cell arteritis (temporal arteritis)
59. Giant cell myocarditis
60. Glomerulonephritis
61. Goodpasture's syndrome
62. Granulomatosis with Polyangiitis (GPA) (formerly called Wegener's Granulomatosis)
63. Graves' disease
64. Guillain-Barre syndrome
65. Hashimoto's encephalitis
66. Hashimoto's thyroiditis
67. Hemolytic anemia
68. Henoch-Schonlein purpura
69. Herpes gestationis
70. Hypogammaglobulinemia
71. Idiopathic thrombocytopenic purpura (ITP)
72. IgA nephropathy
73. IgG4-related sclerosing disease
74. Immunoregulatory lipoproteins
75. Inclusion body myositis
76. Interstitial cystitis
77. Juvenile arthritis
78. Juvenile diabetes (Type 1 diabetes)
79. Juvenile myositis
80. Kawasaki syndrome
81. Lambert-Eaton syndrome
82. Leukocytoclastic vasculitis
83. Lichen planus
84. Lichen sclerosus

85. Ligneous conjunctivitis
86. Linear IgA disease (LAD)
87. Lupus (SLE)
88. Lyme disease, chronic
89. Meniere's disease
90. Microscopic polyangiitis
91. Mixed connective tissue disease (MCTD)
92. Mooren's ulcer
93. Mucha-Habermann disease
94. Multiple sclerosis
95. Myasthenia gravis
96. Myositis
97. Narcolepsy
98. Neuromyelitis optica (Devic's)
99. Neutropenia
100. Ocular cicatricial pemphigoid
101. Optic neuritis
102. Palindromic rheumatism
103. PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus)
104. Paraneoplastic cerebellar degeneration
105. Paroxysmal nocturnal hemoglobinuria (PNH)
106. Parry Romberg syndrome
107. Parsonnage-Turner syndrome
108. Pars planitis (peripheral uveitis)
109. Pemphigus
110. Peripheral neuropathy
111. Perivenous encephalomyelitis
112. Pernicious anemia
113. POEMS syndrome
114. Polyarteritis nodosa
115. Type I, II, & III autoimmune polyglandular syndromes
116. Polymyalgia rheumatica
117. Polymyositis
118. Postmyocardial infarction syndrome

119. Postpericardiotomy syndrome
120. Progesterone dermatitis
121. Primary biliary cirrhosis
122. Primary sclerosing cholangitis
123. Psoriasis
124. Psoriatic arthritis
125. Idiopathic pulmonary fibrosis
126. Pyoderma gangrenosum
127. Pure red cell aplasia
128. Raynauds phenomenon
129. Reactive Arthritis
130. Reflex sympathetic dystrophy
131. Reiter's syndrome
132. Relapsing polychondritis
133. Restless legs syndrome
134. Retroperitoneal fibrosis
135. Rheumatic fever
136. Rheumatoid arthritis
137. Sarcoidosis
138. Schmidt syndrome
139. Scleritis
140. Scleroderma
141. Sjogren's syndrome
142. Sperm & testicular autoimmunity
143. Stiff person syndrome
144. Subacute bacterial endocarditis (SBE)
145. Susac's syndrome
146. Sympathetic ophthalmia
147. Takayasu's arteritis
148. Temporal arteritis/Giant cell arteritis
149. Thrombocytopenic purpura (TTP)
150. Tolosa-Hunt syndrome
151. Transverse myelitis
152. Type 1 diabetes
153. Ulcerative colitis

154. Undifferentiated connective tissue disease (UCTD)
155. Uveitis
156. Vasculitis
157. Vesiculobullous dermatosis
158. Vitiligo
159. Wegener's granulomatosis (now termed Granulomatosis with Polyangiitis (GPA).

INFLAMMATORY DISEASES FOR TREATMENT OR PREVENTION BY THE METHOD

1. Alzheimer
2. ankylosing spondylitis
3. arthritis (osteoarthritis, rheumatoid arthritis (RA), psoriatic arthritis)
4. asthma
5. atherosclerosis
6. Crohn's disease
7. colitis
8. dermatitis
9. diverticulitis
10. fibromyalgia
11. hepatitis
12. irritable bowel syndrome (IBS)
13. systemic lupus erythematous (SLE)
14. nephritis
15. Parkinson's disease
16. ulcerative colitis.

GROWTH PROMOTERS & LOWERING FOOD CONVERSION RATIOS

The Examples demonstrate that target bacteria can be targeted using an antibacterial agent to promote growth and enhance FCR in poultry. In the Example, a guided nuclease system was used to specifically target *Salmonella* in the poultry.

In a first aspect, there is provided:-

A method of promoting the growth of an animal (eg, a livestock animal, eg, a poultry animal), the method comprising administering to the animal a guided nuclease system or a component thereof, and introducing the system or component into target bacteria comprised by the animal, wherein the guided nuclease is capable of recognising and modifying (eg, cutting) a target nucleotide sequence comprised

by the target bacteria, whereby target bacteria are killed or the growth or proliferation of target bacteria are inhibited and the growth of the animal is promoted.

The method is a non-medical method and the presence of target bacteria in the animal is capable of inhibiting the growth of the animal. Thus, the method reduces the burden of such bacteria in the animal and promotes growth.

In a second aspect there is provided:-
A method of enhancing feed conversion ratio (FCR) in an animal (eg, a livestock animal, eg, a poultry animal), the method comprising administering to the animal a guided nuclease system or a component thereof, and introducing the system or component into target bacteria comprised by the animal, wherein the guided nuclease is capable of recognising and modifying (eg, cutting) a target nucleotide sequence comprised by the target bacteria, whereby target bacteria are killed or the growth or proliferation of target bacteria are inhibited and the FCR of the animal is enhanced.

The method is a non-medical method and the presence of target bacteria in the animal is capable of increasing the FCR of the animal. Thus, the method reduces the burden of such bacteria in the animal and enhances FCR (ie, reduces FCR number).

In a third aspect there is provided:-
A method of promoting the growth of an animal (eg, a livestock animal, eg, a poultry animal), the method comprising administering to the animal an antibacterial agent that is toxic to *Salmonella* bacteria, wherein *Salmonella* target bacteria comprised by the animal are exposed to the agent and are killed or the growth or proliferation of target bacteria are inhibited and the growth of the animal is promoted.

The method is a non-medical method and the presence of target bacteria in the animal is capable of inhibiting the growth of the animal. Thus, the method reduces the burden of such bacteria in the animal and promotes growth.

In a fourth aspect there is provided:-
A method of enhancing feed conversion ratio (FCR) in an animal (eg, a livestock animal, eg, a poultry animal), the method comprising administering to the animal an antibacterial agent that is toxic to *Salmonella* bacteria, wherein *Salmonella* target bacteria comprised by the animal are exposed to the

agent and are killed or the growth or proliferation of target bacteria are inhibited and the FCR of the animal is enhanced.

The method is a non-medical method and the presence of target bacteria in the animal is capable of increasing the FCR of the animal. Thus, the method reduces the burden of such bacteria in the animal and enhances FCR (ie, reduces FCR number).

In any of these aspects, optionally the animal is a livestock animal. Optionally, the animal is a bird, eg, a poultry bird, eg, a chicken, turkey, goose or duck. Preferably, the animal is a chicken.

In any of these aspects, optionally the bacteria are *Enterobacteriaciae* bacteria, eg, *Salmonella*. For example, the bacteria are Salmonella enterica, typhimurium or *enteritidis*. For example, the *Salmonella* is any *Salmonella* species or strain disclosed herein.

For example, the system, component or agent is supplied to the animal in an animal feed and/or beverage (eg, mixed in drinking water). When supplied in a beverage, the system, component or agent may be comprised by carrier bacteria, wherein the carrier bacteria are comprised in the beverage at an amount of from 1 x 10^3 to 1 x 10^{10} (eg, from 1 x 10^4 to 1 x 10^{10}; from 1 x 10^4 to 1 x 10^9; from 1 x 10^4 to 1 x 10^8; from 1 x 10^4 to 1 x 10^7; from 1 x 10^3 to 1 x 10^{10}; from 1 x 10^3 to 1 x 10^9; from 1 x 10^3 to 1 x 10^8; from 1 x 10^3 to 1 x 10^7; from 1 x 10^5 to 1 x 10^{10}; from 1 x 10^5 to 1 x 10^9; from 1 x 10^5 to 1 x 10^8; from 1 x 10^5 to 1 x 10^7; from 1 x 10^6 to 1 x 10^{10}; from 1 x 10^6 to 1 x 10^9; from 1 x 10^6 to 1 x 10^8; or from 1 x 10^6 to 1 x 10^7) cfu/ml. When supplied in a beverage, the system, component or agent may be comprised by carrier bacteria, wherein the carrier bacteria are comprised in the beverage at an amount of at least 1 x 10^8 cfu/ml, eg, wherein the animal is a poultry bird, such as a chicken.

Optionally, the guided nuclease is any guided nuclease disclosed herein, eg, a Cas, TALEN, meganuclease or a zinc finger nuclease. In an example, the component is a crRNA or guide RNA that is operable in target cells with a cognate Cas nuclease. The Cas nuclease can be any Cas nuclease disclosed herein. The Cas nuclease may be an endogenous Cas of the target cells or may be encoded by an exogenous nucleic acid that is administered to the animal.

Systems, components and agents of the invention may be introduced into target bacteria by bacterial conjugation (eg, conjugative transfer from a carrier cell to the target cell) or by phage wherein the phage transduce into the target cells nucleic acid encoding the system, component or agent.

Optionally, the target bacteria are comprised by any microbiota disclosed herein that is found in animals. Preferably, the microbiota is a gut microbiota (eg, a gut microbiota of a chicken).

The livestock animal can be any livestock animal disclosed herein, eg, a chicken, pig, cow, sheep, farmed fish (such as salmon or catfish) or farmed shellfsih (eg, lobster, prawn or shrimp).

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine study, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims. All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications and all US equivalent patent applications and patents are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps

The term "or combinations thereof" or similar as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example,

expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, MB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

Any part of this disclosure may be read in combination with any other part of the disclosure, unless otherwise apparent from the context.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

EXAMPLES

Example 1: Target Gene Sequence Assessment & crRNA Array Design

List of putative target genes in *Salmonella*

Through careful analysis and decision making, we compiled the following target genes for assessment by cutting using CRISPR/Cas components delivered by conjugative plasmids from carrier cells as per the invention.

1) pipA Pathogenicity island encoded protein: SPI5
2) mViM putative virulence factor
3) mViN putative virulence factor
4) phoP Transcribes genes expressed under low Mg+ concentration (OmpR family); virulence transcriptional regulatory protein PHOP
5) hilA Invasion genes transcription activator
6) BigA putative surface-exposed virulence protein
7) sugR ATP binding protein; Pathogenicity island encoded protein: SPI3
8) rhuM Pathogenicity island encoded protein: SPI3
9) pipC invasion gene E protein/ Pathogenicity island encoded protein: SPI5
10) pipB Pathogenicity island encoded protein: SPI5

11) sicP chaperone related to virulence
12) sopB invasion gene D protein/Pathogenicity island encoded protein: SPI5
13) marT putative transcriptional regulatory protein/Pathogenicity island encoded protein: SPI3

Target selection

Initial target selection of *Salmonella*-specific genes identified 13 putative candidates located almost exclusively in *Salmonella* pathogenicity islands (listed at the start of this example). After close inspection, the list of targets was reduced to seven genes, namely *pipA*, *pipB*, *pipC*, *hilA*, *marT*, *sicP* and *sopB,* that we found importantly showed no significant sequence homology to other genes from *Enterobacteria*, thus enabling high specificity of targeting Salmonella cells in vivo and in microbiomes containing non-target *Enterobacteria* of different (non-*Salmonella*) species. Target sites for *S. pyogenes* Cas9 defined by presence of the PAM motif (NGG) were extracted using the respective function available in molecular biology software. A number of 6 to 14 putative target sites were identified in each of the seven selected genes.

crRNA design and synthesis

To select target sites most likely to result in efficient restriction of dsDNA, all potential sequences were screened for the presence of nucleotides at key position that have been described to have positive or negative effects on the efficiency of double strand cuts introduced by Cas9 in the available literature. Three publications reported such data, although on sgRNA:

(1) Doench et al. 2014, Nature Biotechnology
(2) Gagnon et al. 2014, PLoS One
(3) Liu et al. 2016, Scientific Reports

One target sequence for each gene that adhered to most of the parameters described influencing restriction efficiency in each of the three publications was selected to be incorporated into a crRNA array, resulting in three arrays to be tested. crRNA arrays were designed based on the sequence data from *S. pyogenes* (published in Deltcheva et al. 2011, Nature). The following sequences were constructed: SEQ ID NOs: 1-3.

Two of these sequences encoding crRNAs were assembled with sequences encoding cognate tracrRNA and Cas9.

Cas9 constructs (tracrRNA-Cas9-crRNA)

The selected sequences encoding crRNAs were combined with different versions of the tracrRNA-Cas9 modules, resulting in a varied set of plasmids.

For constitutive expression of all components (tracrRNA, Cas9 and crRNA), a tracr-Cas fragment based on plasmid pCas9 (Jiang et al. 2013, Nature Biotechnology; sequence presented as SEQ ID NO: 4) was amplified from *S. pyogenes* gDNA. We will join this fragment with the available crRNA-encoding fragments and clone into high copy number general cloning plasmid pJet1.2. This series of construct is named pFS1.

To determine whether expression of this constitutive CRISPR/Cas construct in a low copy number background will work as efficiently, we will move the system described above into a low copy number plasmid. pFS2 constructs are therefore based on the low copy number pSC101 origin from pZS21MCS (Expressys).

To control expression of at least one of the CRISPR/Cas components by means of an inducible promoter, we will replace the constitutive Cas9 promoter with the TetR regulated promoter PLtetO-1. The resulting plasmids of pFS3 are based on the low copy number pSC101 origin of pZS21MCS as well. The introduced changes will be as follows:

- Spacing between RBS and Start ATG has been shortened to 7 bp (TAAATAC)
- Stop codon has been changed from a less effective TGAC to a tandem TAATAAT sequence

Lastly, we will produce a mobilisable version of the constitutively expressed plasmids like pFS1 and pFS2 mentioned above. To this end, we will clone the origin of transfer (oriT) of plasmid RP4 into an intermediate copy number plasmid (p15A origin) carrying a chloramphenicol resistance. This construct should be able to be mobilised and transferred by a chromosomally integrated conjugation machinery as present in RP4 and other mobilisable plasmids.

Further constructs

To determine the efficiency of dsDNA restriction by the selected crRNAs prior to a final selection and transformation into *Salmonella*, we will aim to clone the target genes (or substantial parts thereof) into a compatible plasmid. The plasmid selected is pZA31MCS (Expressys) with a p15A origin compatible to the pFS1/pFS2/pFS3 constructs described above. Efficiency can then be tested in *E. coli* by loss of chloramphenicol resistance (or loss of fluorescence if a yfp derivative is present as a reporter). The best performing target sequences can then be used in future, more targeted plasmids.

We made the following plasmids:-

pFSmob-C	tracrRNA/Cas9 – mob - pZA31MCS
pFSmobSal7	tracrRNA/Cas9 - Salcr7-3 – mob - pZA31MCS
pFSmobSal3	tracrRNA/Cas9 – Salcr3.2 – mob - pZA31MCS
pFSmob-C*	control based on Salcr7-3 – mob - pZA31MCS

Plasmid details

Plasmid Name:	pFSmob-C
Description:	p15A origin plasmid based on pZA31MCS (Expressys) carrying 4768 bp insert (tracrRNA-Cas9-oriT/mob)
Plasmid Length:	6685 bp
Sequence verified:	Yes; Insert sequence verified by primer walking sequencing
Additional Notes:	No crRNA array present; Control plasmid

Plasmid details

Plasmid Name:	pFSmobSal7
Description:	p15A origin plasmid based on pZA31MCS (Expressys) carrying 5598 bp insert (tracrRNA-Cas9-crRNA-oriT/mob)
Plasmid Length:	7515 bp
Sequence verified:	Yes; Insert sequence verified by primer walking sequencing

Plasmid details

Plasmid Name:	pFSmobSal3
Description:	p15A origin plasmid based on pZA31MCS (Expressys) carrying 5341 bp insert (tracrRNA-Cas9-crRNA-oriT/mob)
Plasmid Length:	7258 bp
Sequence verified:	Yes; Insert sequence verified by primer walking sequencing

Plasmid details

Plasmid Name:	pFSmob-C*
Description:	p15A origin plasmid based on pZA31MCS (Expressys)
Plasmid Length:	7348 bp
Sequence verified:	Yes; Insert sequence verified by primer walking sequencing

Example 2: selectively removal of unwanted *Salmonella* strains by conjugation using a guided nuclease

Purpose of the study

The objective of this study was to selectively remove unwanted *Salmonella* strains by conjugation experiment. We mobilised the pFSMobSal7 plasmid from *E.coli* S17 to *Salmonella* spp. This plasmid contains tracr-Cas9 the crRNA-encodng array (Sal7-3) and the origin of transfert (oriT).

Summary

As a proof of concept the conjugation experiment was first done from *E.coli* to *E.coli* before mobilising to *Salmonella* spp. The plasmid pFSMobSal7 (chloramphenicol resistant, CmR) was first transformed into a carrier strain that allows the mobilisation. In this experiment, the strain that produced the pilus was *E.coli* S17 (ATCC® 47055TM). The conjugative functions are provided by an RP4 plasmid integrated into the chromosome. The recipient cell should be Nalidixic acid resistant (25 µg/ml), here we used *E.coli* JM109 (ATCC® 53323TM). The *Salmonella* targeted was FS26 from our *Salmonella* spp collection. The results showed a good efficiency of conjugation from *E.coli* S17 to *E.coli* JM109 and from *E.coli* S17 to *Salmonella enteritidis* (FS26). After conjugation to *Salmonella* as a recipient the killing with pFSMob-3 was efficient.

Introduction

The pFSmobSal7 plasmid was originated from the intermediated copy number pZA31MCS **(Expressys)**. An origin of transfer (OriT) sequence was synthesised (called **mob2)** and cloned into pZA31MCS by assembly method in addition to the tracr-Cas9 and the crRNA array fragments.

Before starting the conjugation experiment, *E.coli* S17 strains were made electro-competent by a standard protocol **(O'Challahan and Charbit, 1990)**. Plasmids pFSMobsal7 and pFSMobC* were then transformed into *E.coli* S17 by electroporation and selected in chloramphenicol (Cm30, 30 µg/mL).

pFSMobSal7 and pFSMobC* were mobilised from the donor *E.coli* S17 to the recipient *E.coli* JM109 and to *Salmonella* spp that are Nalidixic resistant (NalR) and chloramphenicol sensitive (CmS) by mating on a filter followed by an incubation at 37°C. The mixtures were then plated in Cm30, Nal25 and Cm30+Nal25. The transconjugants were selected in double antibiotics Cm30+ Nal25 (Phornphisutthimas et al, 2007).

Note that before starting the conjugation experiment, the efficiency of killing of pFSMob plasmids were first tested by transformation in *Salmonella*.

Methodology

Material:

- Donor strain: *E.coli* S17 transformed with pFSMobSal7 / pFSMobC*
- Recipient strains: JM109 / *Salmonella enteritidis* FS26
- Luria Bertani Broth (LB) (Product number **L3522,** Sigma-Aldrich), LB Cm30 µg/ml
- Plates of LB agar 1%
- Plates of LB agar 1% Cm30 µg/ml
- Plates of LB agar 1% NaL25 µg/ml
- Plates of LB agar 1% Cm30+ NaL25
- Whatman® membrane filters nylon, pore size 0.45 µm to 1 µm, diameter 25 mm. Reference number **28420770** supplied by Sigma-Aldrich

Experiment 1 : Mobilisation of pFSMobC* from *E.coli* S17 to *E.coli* JM109

As a proof of concept, the conjugation experiment was done first from *E.coli* S17 containing a control plasmid (pFSMobC*) to *E.coli* JM109. This experiment followed the protocol from of Phornphisutthimas (Phornphisutthimas et al, 2007):

1. Grow overnight cultures of donors pFSMobC*(S17) from our collection in LB +Cm30 and recipient strain JM109 in LB broth.
2. In the morning pre-warm the plates of LB agar (1%) for at least 30 minutes before starting the experiment
3. Mix the donor strains with the recipient at the ratio of 1:1 as following:
4. 50 µl of pFSMobC*(S17)+ 50 µl of JM109
5. 50 µl of pFSMobC*(S17) + 50 µl of LB
6. 50 µl of LB + 50 µl of JM109
7. For each reaction, put a filter membrane (0,45 µM to 1 µM) on the top of the pre-warmed LB agar plate (1%)
8. Load each sample of mating on a filter
9. Leave the plates 3 hours at 37°C without shaking, the LB will be absorbed and the bacteria should stay on the filter
10. After incubation, put each filter into a universal tube with 1ml LB broth and vortex well
11. Plate 100 µl (And serial dilution) on Cm30, Nal25 and Cm30+ Nal25, leave O.N at 37°C.

The plates of Cm30 give the number of donors. Plates of Nal25 give the number of recipient and plates of Nal25+Cm30 give the number of transconjugants (i.e plasmid mobilised from donor to recipient). Plates with donors and recipient alone (no mating) are negative controls

Results

1) After 18 hours at 37°C colonies were individually counted at different dilutions, when countable, and the number of colonies was expressed as cfu/mL. No colonies were recovered on negative control plates, as expected (Data not shown). The results (Table 1) show a good efficiency of conjugation from *E. coli* S17 to *E.coli* JM109.

Conjugative plasmid	**Number of donors per ml S17 (Cm30)** [a]	**Number of recipient per ml JM109 (Nal25)** [a]	**Number of transconjugants per ml (Cm30+Nal25)** [a]	**Efficiency** [b]
pFSMobC*	$9.46*10^4$	$1.004*10^5$	$2.35*10^4$	$2.4*10^{-1}$

Table 1. Conjugation of pFSMobC* from *E. coli* S17 to *E.coli* JM109. Results obtained from mating on filter after 3 hours of incubation at 37°C. a: All the controls have been checked. b: The efficiency of conjugation is the number of transconjugants per donor cell

2) The mobilisation of pFSmobC* plasmid in the recipient strain was confirmed by PCR. Primers spCas9-6 forward (5'-ATTGTTTGTGGAGCAGCATAAGC)(SEQ ID NO: 8) and mob2 reverse (5'-GCCTCTAGCACGCGTACCATGGGAT) (SEQ ID NO: 9) were used with an annealing temperature of 50°C. Two colonies from plates with transconjugants have been tested by PCR. Positive controls were also included **(Figure 1).**

Conclusion 1:

The conjugation experiment from *E.coli* to *E.coli* has shown a good efficiency of mobilisation. Further optimisation tests on *E.coli* conjugation showed a higher efficiency of conjugation when the mating was incubated for 6 hours at 37°C with a ratio of 1:4.

Experiment 2 : Conjugation of pFSMobSal7 from *E.coli* S17 to *Salmonella* spp

This experiment is following the protocol from of Phornphisutthimas (Phornphisutthimas et al, 2007) and Carraro et al, 2017 with some modifications:

1. Grow overnight cultures of pFSMobSal7 (S17) and the control pFSMobC*(S17) from our collection in LB +Cm30 and recipient strain *Salmonella enteritidis* FS26 in LB broth.
2. In the morning, pre-warm the plates of LB agar (1%) for at least 30 minutes before starting the experiment
3. Mix the donor strains with the recipient at the ratio of 1:4 as following:
4. 100 µl of pFSMobC*(S17)+ 400 µl of *Salmonella* FS26

5. 100 µl of pFSMobSal7(S17)+ 400 µl of *Salmonella* FS26
6. 100 µl of pFSMobC*(S17)+ 400 µl of LB
7. 100 µl of pFSMobSal7 (S17)+ 400 µl of LB
8. 100 µl of LB + 400 µl of *Salmonella*
9. Spin at 2000xg for 3 minutes
10. Resuspend in 200 µl of LB
11. Spin at 2000xg for 3 minutes
12. Resuspend in 25 µl of LB
13. For each reaction, put a filter membrane (0,45 µM to 1 µM) on the top of the pre-warmed LB agar plate (1%)
14. Load each sample of mating on a filter
15. Leave the plates 6 hours at 37°C without shaking, the LB will be absorbed and the bacteria should stay on the filter
16. After incubation, put each filter into a universal tube with 1ml LB broth and vortex well
17. Plate 100 µl (And serial dilution) on Cm30, Nal25 and Cm30+ Nal25, leave O.N at 37°C.

The plates of Cm30 plates give the number of donors. Plates of Nal25 give the number of recipient and plates of Nal25+Cm30 give the number of transconjugants (i.e plasmid mobilised from donor to recipient).

Results

- After 18 hours at 37°C colonies were individually counted at dilution -3, and the number of colonies was expressed as cfu/mL (Table 2). No colonies were recovered on negative control plates, as expected (Data not shown).

Conjugative plasmid	Donor S17/ml (Cm30)	Recipient FS26/ml (Nal25)	Number of transconjugants	Number of transconjugants/ml (Cm30+Nal25)	Conjugation efficiency[a]
pFSMobC*	$6.6*10^6$	Full	72	$7.2*10^5$	$1.09*10^{-1}$
pFSMobSal7	$4.7*10^6$	Full	0	0	0

Table 2: Conjugation experiment results of pFSMob plasmids from *E.coli* S17 to *Salmonella enteritidis* FS26. Results obtained from mating of a filter for 6 hours at 37°C.

- In comparison with the plasmid control pFSMobC*, these data show that the plasmid pFSMobSal7 delivered to the target strain by conjugation is able to remove by 100% the strain *Salmonella enteritidis* (FS26) (Figure 2).

Conclusion

The study showed that the conjugation is a good method of delivery from *E.coli* S17 to *Salmonella enteritidis* (FS26) and that pFSMobSal7 is still efficiently killing when delivered to the recipient by conjugation. Conjugation was able to remove (kill) 100% of the *Salmonella.*

References

Carraro N, Durand R, Rivard N, Anquetil C, Barrette C, Humbert M, Burrus V. (2017). *Salmonella* genomic island 1 (SGI1) reshapes the mating apparatus of IncC conjugative plasmids to promote self-propagation. PLOS Gen.

O'Challahan D, Charbit A. (1990). High efficiency transformation of *Salmonella typhimurium* and *Salmonella typhi* by electroporation. Mol. Gen. Genet., 223;156-8.

Phornphisutthimas S, Thamchaipenet A, and Panijpan B. (2007). Conjugation in Escherichia coli. Biochem and Mol Bio Education Vol. 35, No. 6, 440-445

<u>Example 3: Pan-serotype conjugative transfer and killing of *Salmonella*</u>

Purpose of the study

The purpose of this study was to test the *in vitro* ability of pFSmobSal7 and pFSmobSal3 plasmids to selectively remove *Salmonella enterica* subsp. *enterica* serotypes by conjugation.

Summary

Eighteen strains of *Salmonella enterica* subsp. *enterica*, serovars Typhimurium (4), Enteritidis (1), Virchow (1), Montevideo (1), Heidelberg (1), Hadar (1), Binza (1), Bredeney (1), Infantis (1), Kentucky (1), Seftenberg (1), Mbandaka (1), Anatum (1), Agona (1) and Dublin (1), were selected from our *Salmonella* spp. collection **(Table 3)**. Bacteria were made competent and transformed with two versions of pFSmob plasmid, i.e. pFSmobSal7 and pFSmobSal3, two versions of pFSmob control plasmid, i.e. pFSmob-C* and pFSmob-C, and pZA31MCS **(Expressys)** as further control. After electroporation and 2hrs of incubation in SOC Outgrowth Medium **(NEB, B90205, LOT-10019857)** at 37°C, different dilutions of the original solution were plated out on 1% LB agar supplemented by chloramphenicol

(Cm_{30}, 30µg/mL). Plates were incubated at 37°C for 18hrs. Results were interpreted as colony forming unit/mL (CFU/mL).

Introduction

The pFSmobSal7 and pFSmobSal3 plasmids carry on their crRNA arrays 7 target genes (*pipA, pipB, pipC, hilA, sicP, mart, sopB*), and 3 target genes (*pipC, hilA, mart*), respectively. Positive control pFSmob-C originated from pFSmobSal7 plasmid was used, in particular pFSmob-C does not contain the crRNA array. Control pFSmob-C originated from the intermediated copy number pZA31MCS **(Expressys)** used as a further control. Selection of positive colonies was performed on chloramphenicol (Cm_{30}, 30µg/mL). It was previously showed the *in vitro* ability of pFSmobSal7 to selectively remove by transformation *Salmonella enterica* serovar Typhimurium, Enteritidis, Virchow, Montevideo, Hadar and Binza. The purpose of this study was to assess all the *Salmonella* spp. serotypes present in our collection, in order to evaluate the *in vitro* ability of both pFSmobSal7 and pFSmobSal3 plasmids to selectively unwanted bacteria; in order to collect significant results, 18 different serotypes were selected from our collection and tested by electroporation **(Table 3)**. Plasmids pFSmob-C, pFSmob-C* and pZA31MCS **(Expressys)** were used as controls.

Methodology

Bacterial strains were recovered from frozen stock kept at -80°C, and cultured at 37°C for 24hrs **(Edwards and Ewing, 1986)**. Susceptibility to Cm_{30} (30µg/mL) was tested using an agar dilution method in accordance with EUCAST clinical breakpoint tables. Bacteria were made competent using O'Challahan and Charbit protocol **(O'Challahan and Charbit, 1990)**. All the selected strains were transformed with 100ng of pFSmobSal7, pFSmobSal3, pFSmob-C, pFSmob-C*, and pZA31MCS **(Expressys)**; a negative control was also included (competent cells without the addition of DNA) **(Table 4)**. After 18hrs of incubation, countable colonies (number of colonies between 30 and 300) were expressed as number of colonies per mL (CFU/mL) **(Table 4).**

Collection no	Salmonella sertypes
FS2	Typhimurium
FS3	Typhimurium
FS10	Typhimurium
FS11	Typhimurium
FS11	Typhimurium

FS26	Enteritidis
FS35	Virchow
FS38	Montevideo
FS41	Heidelberg
FS42	Hadar
FS43	Binza
FS44	Bredeney
FS50	Infantis
FS58	Kentucky
FS60	Seftenberg
FS61	Mbandaka
FS62	Anatum
FS64	Agona
FS67	Dublin

Table 3. *Salmonella* spp. collection. Eighteen strains of *Salmonella enterica* subsp. *enterica*, serovars Typhimurium (4), Enteritidis (1), Virchow (1), Montevideo (1), Heidelberg (1), Hadar (1), Binza (1), Bredeney (1), Infantis (1), Kentucky (1), Seftenberg (1), Mbandaka (1), Anatum (1), Agona (1) and Dublin (1), were selected from our *Salmonella* spp. collection.

Results

All the bacteria selected were found to be susceptible to Cm_{30} according to EUCAST breakpoints **(EUCAST, 2013)**. After electroporation and plating of different dilutions (undiluted, 10^{-1}, 10^{-2}) on LB agar plates **(Fisher Bioreagents, BP1425-500, LOT-171784)** supplemented with Cm_{30} (30µg/mL) **(Sigma)**, plates were incubated for 18 hrs at 37°C. Bacteria were considered transformed when they grew on Cm_{30} (30µg/mL) agar plates. Countable colonies (between 30 and 300) were expressed as CFU/ml (**Table 4**). No colonies were recovered on negative control plates, as expected. All the serotypes but one (FS67, *S.* Dublin) were efficiently transformed with pZA31MCS **(Expressys)**. Almost all the serotypes were efficiently transformed with the two control plasmids (pFSmob-C* and pFSmob-C) with a number of colonies between 10^2 and 10^6 CFU/mL.

With regard to pFSmobSal7 and pFSmobSal3, the killing effect occurred in all the serotypes and it was found to be similar. In the majority of the serotypes, a decreasing in the number of colonies was relatively evident in comparison to the ones transformed with the control plasmids. No significant

differences were found between pFSmobSal7 and pFSmobSal3 in their killing effect, except in one strain (FS38, *S.* Montevideo).

Collection	Serotype	pZA31MCS	pFSmob-C*	pFSmob-C	pFSmobSal7	pFSmobSal3
FS2	Typhimurium	4.8*10^4	1*10^5	4*10^4	0	6*10^1
FS3	Typhimurium	5*10^5	1*10^5	3*10^3	1*10^1	6*10^1
FS10	Typhimurium	5*10^5	0	1.1*10^2	0	0
FS11	Typhimurium	4.7*10^4	7*10^2	4.1*10^2	0	0
FS26	Enteritidis	>1*10^6	9*10^5	>1*10^6	3*10^1	4*10^1
FS35	Virchow	>1*10^6	2.8*10^3	1.3^10^4	0	0
FS38	Montevideo	>1*10^6	2.1*10^4	4.2*10^4	1.4*10^2	0
FS41	Heidelberg	1.1*10^4	4.1*10^2	6*10^2	0	0
FS42	Hadar	>1*10^6	7*10^2	0	0	0
FS43	Binza	1.7*10^4	7*10^1	1*10^1	0	0
FS44	Bredeney	>1*10^6	1*10^5	9*10^4	1.7*10^3	3*10^3
FS50	Infantis	1*10^4	1.8*10^2	2*10^3	0	4*10^1
FS58	Kentucky	1.7*10^4	7.5*10^3	3.6*10^3	0	0
FS60	Seftenberg	>1*10^6	1.2*10^5	>1*10^5	2*10^1	2.4*10^2
FS61	Mbandaka	2*10^2	2*10^1	0	0	0
FS62	Anatum	1.4*10^3	4*10^1	0	0	0
FS64	Agona	>1*10^6	>1*10^6	3*10^4	7.8*10^2	3*10^3
FS67	Dublin	NT*	NT	NT	NT	NT

Table 4. Transformation of 18 *Salmonella* spp. serotypes. Eighteen *Salmonella* spp. serotypes were transformed with pZA31MCS **(Expressys)**, pFSmob-C*, pFSmob-C, pFSmobSal7, and pFSmobSal3. Almost all the serotypes were efficiently transformed with pZA31MCS **(Expressys)**, pFSmob-C*, and pFSmob-C, with some exceptions. No significant differences were observed in the transformation with pFSmobSal7 and pFSmobSal3. Strain FS67, *S.* Dublin, was found to be not transformable (NT*) with all the plasmids used in this study.

Conclusion

All the *Salmonella* spp. serotypes used in this experiment were efficiently made competent and transformed with pZA31MCS **(Expressys)**, pFSmob-C and pFSmob-C* used as controls. *Salmonella Typhimurium* serotype seems not to be always transformable by pFSmob-C*, as observed previously. More in general, no particular differences occurred between the two pFSmob-derived control plasmids used in this study (between 10^2 and 10^6 CFU/mL). On the other hand, the

killing effect of pFSmobSal7 and pFSmobSal3 was found to be similar (between 10^1 and 10^3). In conclusion, in this study it was demonstrated that pFSmobSal7 and pFSmobSal3 can selectively remove different *Salmonella* spp. serotypes in comparison with the control plasmids.

Reference

Edwards PR, Ewing WH. (1986). Identification of Enterobateriaceae (4th ed.). Elsevier, New York.

EUCAST, European Committee on Antimicrobial Susceptibility Testing. (2013). Breakpoint tables for interpretation of MICs and zone diameters. *Available at: http://www.eucast.org (last accessed August 16, 2013.*, Version 3.1.

O'ChallahanD, Charbit A.(1990). High efficiency transformation of *Salmonella typhimurium* and *Salmonella typhi* by electroporation. Mol. Gen. Genet., 223;156-8.

Example 4: Feed Conversion Ratio Improvement in chickens. Combined second 2 week efficacy, 2 week safety and 6 week productivity trial.

Purpose of study

- To determine the effect antibacterial conjugative plasmids (pFSmobSal7) on the health, welfare and productivity of chickens over 6 weeks.

Methodology

Ross 308 birds were housed under controlled biosecure conditions and given water and standard commercial rations *ad libitum*.

Birds were dosed continually with either:

1. No addition to water (60 birds)
2. Strain S17 @ 10^8 cfu/ml drinking water (30 birds)
3. Strain S17 containing pFSmob-C* @ 10^8 cfu/ml drinking water (30 birds)
4. Strain S17 containing pFSmobSal7 @ 10^8 cfu/ml drinking water (60 birds)
5. Strain S17 containing pFSmobSal7 @ 10^9 cfu/ml drinking water (30 birds)
6. Strain S17 containing pFSmobSal7 @ 10^{10} cfu/ml drinking water (30 birds)

Bacterial strains were recovered from frozen stocks kept at -78C and cultured on LB agar for 24 h at 37C. Cultures were prepared daily in LB broth containing antibiotic to prevent loss of plasmid with shaking at 180 rpm for 16h at 37C then centrifuged for 10 min at 4000 x *g*. Medium was removed and the pellet resuspended in PBS then diluted in PBS to give solution for dosage to chickens.

In parallel, a group of 30 birds was dosed orally with 0.5 ml 10^5CFU/mL *Salmonella* Enteritidis strain FS26 (Folium) on day 1. Birds were checked for *Salmonella* colonisation at day 3 of the experiment by cloacal swab using ISO 6759 methods (1). On day 5 of the experiment 3 of these verified *Salmonella*-colonised birds (seeder birds) were marked and added to each of groups 1-6. Birds were weighed weekly and feed consumption and mortality recorded.

Fifteen birds from each group were euthanised on days 12 and 19 (7 and 14 days post mixing with seeder birds). Caeca were removed for examination for *Salmonella* by ISO 6759. Hock and pad marks were recorded. Samples of 1g liver and caecal contents were snap frozen.

Thirty birds from each of group 1 and 4 were then monitored for behaviour and weight weekly until day 42, when they were euthanised and examined as for birds above.

Results were recorded on paper or dictated via telephone or radio in biosecure accommodation and transcribed to Microsoft Excel™. For the purposes of data transformation birds with no bacteria detected were allocated a count of 1 bacterium per g. Counts and weight were log transformed and statistical analysis conducted using GraphPad Prism™. Data was assessed for normality of distribution using a D'Agostino and Pearson omnibus normality test and as non-normal was analysed using a Kruskall-Wallis test with Dunn's multiple comparison test post hoc. Differences in proportions of birds was analysed using Fisher's exact test.

Results

All seeder birds were colonised with *Salmonella* by day 3. Those used to seed infections in the test groups had counts of $>10^5$ cfu/g faeces.

At day seven 15 birds per group were euthanised and *Salmonella* in the caecum enumerated (Figure 3). Figure 4 shows data presented a birds positive/negative for *Salmonella*.

Birds dosed with S17-pFSmobSal7 (lowest dose) and pFSmob-C* had significantly lower numbers of *Salmonella* in the caecum than controls (Figure 3) and were significantly less likely to have detectable *Salmonella* in the caecum at day 7 post mixing with seeder birds.

No mortality was seen in any group; no morbidity was observed and high dosage of S17-mobSal7 was tolerated well. Birds were scored for positive and negative behaviours, but no difference was seen between groups.

Thirty birds from each of groups 1 and 4 were monitored further until 42 days of age. During this period again, no significant differences in behaviour were observed. Feed consumption between groups was not significantly different and weights did not differ significantly between groups measured in life. At post mortem *Salmonella* was not detectable in the caecum by direct count; no hock or pad marks were observed. Median carcass weight of birds in the S17-pFSmobSal7 group was 205 g greater than in the control group, a significant increase (Figure 5). As this was associated with equivalent feed consumption between groups, the S17-pFSmobSal7 group had a significantly improved feed conversion ratio (FCR).

References

ISO. 2007. ISO 6579:2007: Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp. (ISO 6579:2002+Amd 1:2007). Geneva, Switzerland

Example 5: Non-replicative conjugative plasmid with conditionally essential gene marker

To meet regulatory requirements, any plasmid used will preferably be devoid of an antibiotic selection marker and will be non-replicative in the vast majority, if not all, cells except for the host cell (ie, carrier cell). To render the plasmid non-replicative, the replication system of broad host range plasmid RK2, a fairly low copy number plasmid, will be utilised. RK2 replication is dependent on the presence of a replication protein, TrfA encoded by trfA on RK2, and binding of TrfA to the vegetative origin (oriV) of the plasmid and does not utilize the host machinery for replication initiation. Physical separation of trfA from the plasmid and incorporation of this gene into the chromosome will make the resulting plasmid carrying RK2 oriV dependent on a host encoded function. This will prevent the plasmid from actively replicating in a target strain after delivery via conjugation. In the rare case of an identical IncP plasmid being present in the target cell that will provide trfA, both plasmids will compete for the available TrfA, resulting in loss of one plasmid. As our plasmid in this example

further does not carry an advantageous antibiotic resistance marker or similar and lacks plasmid addiction systems, it will be quickly lost from the offspring.

As a selection marker in the absence of an antibiotic resistance, the *aroA* gene encoding for an enzyme in the biosynthesis pathway of aromatic amino acids will be used. This is a conditionally essential gene that is essential when the host cell is grown in the absence of available aromatic amino acids or an intermediate from the reaction catalysed by *aroA*. Moving the *aroA* gene from the host genome to the plasmid backbone will provide this selection marker. The *aroA* gene of the chromosome will be replaced in the host (carrier cell) strain by a copy of *trfA*.

An *aroA* knock-out and replacement of the chromosomal copy by a trfA expression cassette in a *E. coli* lab strain DH10B will be made to generate a test strain of bacteria for testing the plasmid. The trfA expression cassette to be used has the sequence of SEQ ID NO: 10.

The final host (carrier) strain, a commensal *E. coli* isolate from chicken, will undergo the same strain construction procedure.

The *aroA* coding sequence (SEQ ID NO: 11) will be amplified with a similar promoter and terminator region and assembled into the plasmid along with modules for the Cas9, tracrRNA, crRNA and the oriV of RK2.

Additionally, alternative modules can be tested in this plasmid configuration such as anti-restriction genes that inhibit Type I restriction enzymes (ocr of T7, klcA of RK2, ardA from conjugative plasmids/transposons, ardB from conjugative plasmids) to improve DNA stability after transfer into target cells.

Further or alternatively, a module encoding an essential part of the type IV secretion system present for conjugation will located on the plasmid as this will lead to a non-functional transfer system present in the cell if the plasmid is not present. To facilitate conjugation by the host cell, the type IV secretion system present on plasmid RK2 will have to be integrated into the host genome. The essential components of this system are encoded within three operons on two locations of the plasmid, tra1 and tra2. Tra1 encodes the the traKLM and traJXIHGF operons, tra2 the trbBCEFGHJL genes. During the integration process, the regulatory region present in tra1 located between the traKLM and traJXIHGF operons will be modified. This sequence contains the origin of transfer (oriT), harbouring the binding site for the TraJ protein responsible for initiating transfer of the DNA. If this binding site

is not altered by mutagenesis to prevent TraJ binding, transfer of chromosomal DNA fragments during the conjugation process will be initiated, leading to transfer of unwanted genetic information.

Sequences of the RK2 tra1 and tra2 modules to be used are in SEQ ID NOs 13 and 14.

Example 6: Anti-*Salmonella* Plasmid Construction & Testing

Summary

The type I-E Cas system from *E. coli* K12 (MG1655) is a RNA guide-directed DNase machinery, known as Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) and CRISPR-associated proteins system (CRISPR-Cas), which represents an adaptive immune system for prokaryotes that targets invading foreign genetic material for degradation. The *Escherichia coli* type I-E Cascade system is made up of different Cas proteins (casABCDE12 and cas3) and recognizes a wide variety of PAM sequences with varying degrees of efficacy. The main PAM sequences are: 5'-AAG, AGG, ATG, GAG-3'. Type I-E Cas system from *E. coli* K12 was used to selectively remove *Salmonella* species. The system was modified as in *E. coli* MG1655 the main components of the cas protein complex are expressed from two transcription units, cas3 and casABCDE12, respectively. The modifications introduced during the plasmid construction process included the exchange of the native, regulated promoter element of the casABCDE operon with the constitutive J23114 promoter and replacing the cas1 and cas2 genes with the cas3 gene including its native ribosomal binding site. These changes, in effect, created a constitutively expressed CRISPR-Cas module that was subsequently tested for functionality by addition of a crRNA array for specific targeting of *Salmonella* species.

Two versions of Guided Biotic® plasmids were constructed differing in origin of replication (oriV) and antibiotic selection marker. The resulting plasmids were named pFS-Sal-08-rm and pFS-Sal-09-rm and were tested *in vitro* to selectively inhibit growth of *Salmonella enterica* subsp. *enterica* serovar Enteritidis by conjugative DNA transfer. In the conjugation experiments, pFS-EcoCas3-01-rm and pFS-EcoCas3-03-rm were used as control plasmids for *Salmonella*-specific growth inhibition

Plasmids were constructed, each comprising nucleotide sequenes encoding Type I-E *E coli* Cas3 and cognate casA, B, C, D and E; a CRISPR array that is operable with the Cas3 and comprises a first spacer that is complementary to a sequence of an *invB* gene of *S enterica*, a second spacer that is complementary to a sequence of a *sicP* gene of *S enterica* and a third spacer that is complementary to

a sequence of a *sseE* gene of *S enterica*; an RP4 oriT; a p15A ori; an *E coli proA* gene; and an *E coli proB* gene. See Table 7 for more details.

Methodology

crRNA array design

Target gene selection, to design the CRISPR spacers, was focused on the *Salmonella*-specific pathogenicity islands (SPIs), which are major virulence factors for *Salmonella*. Criteria for selection were conservation within *Salmonella enterica* and a low to very low occurrence and conservation of the respective gene in other bacteria. These analyses were performed using the Basic Local Alignment Search Tool (BLAST). Cut-off for number of hits in non-*Salmonella* species was set at 1000. Level of conservation was considered as high if a search produced >1000 hits. Between 100 and 1000 was medium, between 20 and 100 low, very low if <20 hits. Specifically, genes associated with the type III protein secretion systems, T3SS, of SPI-1 and SPI-2 were explored as targets, taking care to include gene candidates that are conserved across *Salmonella* species but exclude those that are conserved across other bacteria species. Initially, the search focused on secreted effectors and chaperones, avoiding regulators and functional components of the T3SS.

The three selected highly conserved gene targets *sicP, invB* and *sseE* are all part of the T3SS loci encoded by *Salmonella* pathogenicity islands (SPIs), SPI-1 in the case of *sicP* and *invB* (both chaperones), and SPI-2 in the case of *sseE* (secreted effector). They all occur in only one copy in *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2 and this is the case for all *Salmonella enterica* although their duplication in some strains cannot be ruled out.

Candidate target genes (a) and, subsequently, spacers (b) were searched on Blast to make sure they are specific for *Salmonella* and to minimize off-targets. Search was performed using RefSeq Database, which contains around 10600 *Salmonella enterica* genomes:

a) Blast search parameters for gene selection:
- *Salmonella* conservation: RefSeq DB restricted to *Salmonella enterica* (taxid:28901), megablast, max hits: 20000, standard parameters
- Occurrence outside *Salmonella*: RefSeq DB restricted to bacteria (taxid:2) excluding *Salmonella* (taxid:590), Blastn, max hits 1000, standard parameters

b) Blast search parameters for spacer sequences (including PAMs):
- *Salmonella* conservation: RefSeq DB restricted *to Salmonella enterica* (taxid:28901), blastn, max hits:20000, standard parameters

- Occurrence outside *Salmonella*: RefSeq DB restricted to bacteria (taxid:2) excluding *Salmonella enterica* (taxid:28901), Blastn, max hits 1000, standard parameters but expect value set to 100 to catch sequences with low but still significant homology

A number of spacers targeting different *Salmonella enterica* genes were initially screened *in silico*, selected, cloned and individually tested *in vitro* for their efficacy of targeting *Salmonella enterica* subsp. *enterica* serovar Enteritidis FS26 (see Summary on target gene and spacer selection and Rep-21: Construction and in vitro testing of spacer reporter systems). The three best performing spacer sequences active against *Salmonella* species were selected and combined into a short crRNA array. The spacer sequences were derived from three highly conserved genes present in *Salmonella* pathogenicity islands (SPIs), *invB, sicP* and *sseE*. In brief, a limited potential spacer sequences characterized by the presence of a strong consensus 5'-PAM sequence capable of initiating DNA restriction were selected and ranked according to sequence features associated with efficient target DNA restriction, conservation within *Salmonella* isolates and lack of DNA homology outside of *Salmonella enterica*. Three spacer sequences meeting these criteria were finally selected and incorporated into a functional crRNA array. The finalised crRNA array was ordered as a synthetic gene sequence inserted into a standard cloning plasmid (GeneArt, Thermo Fisher Scientific).

Sequences of selected genes are shown in SEQ ID NOs: 20-22. In each gene, spacers are highlighted in bold and respective PAMs in italics (in the case of *invB*, the spacer was selected on the negative DNA strand (anti-sense) so the PAM appears 3' to the spacer. PAM sequences used for spacer selection were AAG, ATG, AGG and GAG. Blast searches for the spacers with their respective PAMs was conducted (Table 10).

The non-*Salmonella* hits were further analysed for their conservation level (spacers accepted when level of conservation in off-targets was <75%). Sequence selection also depended on where the mismatches were located in the off-target hit, ruling out the ones showing conserved PAM and seed (first 8pb of the spacer). One additional criterion for our selection also included the type of bacteria where the hit was present, if present in a pathogen, the spacer was still considered as a possible candidate.

Each spacer was individually tested *in vitro* for their efficacy of targeting *Salmonella enterica* subsp. *enterica* serovar Enteritidis FS26, before combining them together in the array present in the plasmid pFS-Sal-09-rm.

Plasmid assembly

For the homology-based DNA assembly, individual DNA modules were carrying 5' and/or 3' extensions with exact homology to the adjacent module. The extensions were either introduced during a PCR amplification step of the module or were incorporated during the design of synthetic DNA modules. The constitutive J23114 promoter was introduced.

CasA-E: The 4464 bp casA-casB-casC-casD-casE module was amplified from *E. coli* K12 (MG1655) genomic DNA.

Cas3: The 2709 bp Cas3 module was amplified.

crRNA: The crRNA array module Sal-crRNA 1 (546 bp), carries a 25 bp homology extension to the 3'-end of the Cas3 module. The sequence of the Sal-crRNA Array1 is shown in SEQ ID NO: 15, with the -10 region of the promoter in italic, direct repeats underlined and the spacer regions corresponding to the selected *Salmonella* target sequences of *invB, sicP* and *seeE*, in bold.

pFS-Sal-08-rm and pFS-Sal-09-rm validation and *in vitro* testing by Conjugation

Having confirmed the sequences of both constructs, the plasmids were transformed into a carrier strain that allowed plasmid mobilization via conjugation to assess the efficacy of the Cas3 system to selectively kill *Salmonella* in presence of the Sal-crRNA array 1. In this study, *E. coli* strain S17-1 ΔTn7 was used. 100 ng of pFS-Sal-08-rm and pFS-Sal-09-rm were used for transformation of 50 µl electrocompetent cells of *E. coli* S17-1 ΔTn7 by electroporation and selected on Kanamycin and Chloramphenicol, respectively. Transformation with both plasmids resulted in a good transformation efficiency of *E. coli* S17-1 ΔTN7 (10^5 -10^6 transformed CFU/mL). Control plasmids, pFS-EcoCas3-01-rm and pFS-EcoCas3-03, lacking the crRNA array, were also transformed in the S17-1 ΔTn7 carrier strain to be used as controls in FS26 transformation for pFS-Sal-09-rm and pFS-Sal-08-rm. In order to confirm the mobilization capability of the new plasmids, S17-1 carrier cells, were first used to conjugate *E. coli* JM109 cells (nalidixic acid, Nal, resistant). Results, reported in Table 8, show a similar conjugation efficiency between pFS-EcoCas3-01 and pFS-Sal-09-rm and between pFS-EcoCas3-03 and pFS-Sal-08-rm, in *E. coli* JM109. Conjugation efficiency is calculated dividing the number (CFU/mL) of transconjugants (selected on plates with double antibiotics) by the number of recipients (selected on plate with Nal).

Subsequently, *E. coli* carrier strain S17-1 ΔTn7, previously transformed with the plasmids, were used to assess whether there is a significant growth inhibition of *Salmonella* Enteritidis FS26 by conjugation of pFS-Sal-08-rm compared to the control plasmid pFS-EcoCas3-03-rm and of pFS-Sal-09-rm compared to pFS-EcoCas3-01-rm. Conjugation was performed from *E. coli* S17-1 ΔTn7 into nalidixic acid (Nal) resistant *Salmonella* Enteritidis FS26. Results showed a significant reduction

(CFU/mL) in *S.* Enteritidis strain FS26 conjugated with both pFS-Sal-08-rm and pFS-Sal-09-rm compared to the respective control plasmids (Table 9).

In conclusion, inhibition of *Salmonella* growth by both plasmids carrying the Sal-crRNA array 1 generated in this study indicated that the modified Cas module is functional and was able to express all the individual components of the Type I-E Cascade complex, the Cas3 nuclease and the crRNA array without negatively affecting growth of non-target *E. coli* host strains S17-1 and JM109. We demonstrated the ability of the *E. coli* type I-E Cas system-based plasmids to be transferred by conjugation from the *E. coli* S17-1 carrier strain to *S.* Enteritidis strain FS26. We observed a significant inhibition of growth in the *Salmonella* transconjugants (>99.9%) of the selected strain through conjugation of both pFS-Sal-08-rm and pFS-Sal-09-rm in comparison to the control used. These data therefore highlighted the potential of these *E. coli* type I-E Cascade based constructs for the removal of unwanted bacteria, such as for zoonotic control.

Example 7: Test of stability and efficacy of a Folium *E. coli*-based product in chickens fed a diet containing *Salmonella*.

In Example 4, we tested a Guided Biotic® plasmid contained in *E coli* carrier cells of S17 strain. In this present Example 7, we instead tested a Guided Biotic® plasmid (GB plasmid pFS-Sal-09-proAB-rm, Example 6 & Table 7) contained in a different strain (Strain X) of *E coli* carrier. A helper plasmid pCon_aroA carried a functional copy of the conjugation machinery of plasmid RP4 (*traJXIHGF-traKLM-trbBCDEFGHIJKL*) that was found to facilitate mobilization of oriT-containing plasmids from the host cell to a recipient cell *in vitro.*

Purpose of study

- To determine the stability of *E. coli* Strain X with the Guided Biotic® in drinking water over 24 hours.
- To enumerate intestinal and organ contamination with *Salmonella* given in-feed.
- To determine the efficacy of *E. coli* Strain X containing an active GB plasmid in chickens challenged with *Salmonella* in-feed.

Summary

- *E. coli* Strain X was found at the expected level of 5 x log-8 CFU/mL immediately after dosing into deionised water containing stabilizers, and 24 hrs had dropped to 8 x log-7 CFU/mL.

- Following challenge in-feed (10^4 CFU/g feed) for 24 hrs, low levels (~log-2-5 CFU/g) of *Salmonella* were found in the crop and caeca on days 1, 3 and 7 after challenge. Lower levels (~log-1 CFU/g) were also found in ileal contents, liver and spleen 7 days post-challenge.
- The active Guided Biotic®, *E. coli* Strain X containing an active GB plasmid, reduced ($P<0.03$) Salmonella counts in the crop 7 days post-challenge by ~log-1 CFU/g.

Methodology

Ross 308 birds (30) were housed under controlled biosecurity conditions and given water and a standard commercial ration *ad libitum*. Each experimental group was held in a separate pen with wood shaving bedding. Birds had a 18 h light / 6 h dark lighting regime. Temperature and humidity were kept between the standard levels shown in Table 11.

Treatment groups:

- Low Control - no addition to water, 10^4 CFU *Salmonella* per gram feed for 24 hrs on day 7 (15 birds)
- GB-Sal - *E. coli* Strain X Guided Biotic® containing active GB plasmid (pFS-Sal-09-proAB-rm) at 10^8 CFU/mL in drinking water from days 1-14, 10^4 CFU Salmonella per gram feed for 24 hrs on day 7 (15 birds)

Guided Biotic® strains were recovered daily from frozen stocks kept at -78°C and cultured on LB agar plus Chloramphenicol (30µg/mL) for 24 h at 37°C. Strain X with active GB plasmid strains were prepared in Terrific broth, grown with shaking at 180 rpm for 16h at 37°C. After measuring OD_{600} to estimate cell numbers, cells were centrifuged and resuspended in deionised water containing stabilizers to give solutions for dosage to chickens (10^8CFU/mL in drinking water). Vac Pac containing blue dye was included 3 hrs before collection on sampling days, to confirm water and GB intake. Supplemented water samples were collected on GB addition and 24 hrs later on days 4-14 of the trial, for enumeration for GB. Water samples were decimally diluted in PBS and then plated on to MacConkey agar number 3 (Oxoid CM0115), then incubated at 24 h at 37°C.

Salmonella strain (FS26, with Nalidixic acid marker) was recovered from frozen stocks kept at -78°C and cultured on LB agar for 24 h at 37°C. Test cultures were prepared daily in LB broth grown with shaking at 180 rpm for 16h at 37°C. After measuring OD_{600} to estimate cell numbers, cells were centrifuged and resuspended and diluted in phosphate-buffered saline to give solutions of $1x10^7$ CFU/ml which was dripped on to the feed at a rate of 1 ml bacterial suspension to 100 g feed while

the feed was mixed thoroughly. Four feed samples were collected for enumeration of *Salmonella* content.

Animal sample collection and plating

No bird mortality was recorded. Lighting level, additions of bedding, temperature, humidity and stocking density were logged and did not differ between treatments. Three birds from each group on days 1 & 3 and nine birds on day 7 after challenge were euthanised and samples taken from the crop, ileal, caecal, liver and spleen. Euthanasia, post-mortem and dissection times were recorded. Feathers were spray wetted with water. Birds were opened with a disposable scalpel that was discarded after opening and gloves were changed. Samples of 1g liver and crop, ileal and caecal contents were taken and snap frozen in liquid nitrogen. Scalpels were disposed of as each organ was taken. Samples were homogenised in 9 volumes of phosphate-buffered saline, decimally diluted in phosphate-buffered saline and examined, with agars incubated for 16-18h at 37°C. Samples that were negative in direct plating were examined for *Salmonella* by enrichment in 9 volumes of selenite cystine broth (Oxoid CM0699) for 16-18h at 37°C. The enriched broth was subsequently streaked on to XLD agar (CM0469) containing antibiotics as Table 12 and incubated at 37°C for 16-18h.

Birds with no bacteria detected in direct counts were allocated a count of 0 bacterium per g, while those negative in direct counts but positive in the enhanced method were allocated 50 CFU/g. Results were recorded on paper or dictated via telephone or radio in biosecure accommodation and transcribed to Microsoft Excel. Data were analysed in GraphPad Prism. Data were assessed for normality of distribution using a D'Agostino and Pearson omnibus normality test and as non-normal was analysed using a Mann-Whitney U test. Paired data were analysed using a paired T-test.

RESULTS

1. ***Salmonella* in-feed**
   - The presence of *Salmonella* in-feed (average 9 x log-4 CFU/g) was as expected.

2. **Guided Biotic® levels in drinking water**
   - The analysed GB level in drinking water immediately on mixing (average 5 x log-8 CFU/mL) was as planned. Samples taken 24 hrs later confirmed an average drop to 8 x log-7 CFU/mL.

3. **Effects of *Salmonella* dose**

- Overall, low levels of *Salmonella* were found in the crop (~log 1-3 CFU/g) and caeca (~log 4-5 CFU/g) on days 1 and 3 after challenge. No *Salmonella* was found in ileal, liver or spleen samples at these times.
- At day 7 post challenge similar levels of *Salmonella* were again found in the crop and caeca, as well as lower levels (~log 1) in the ileum, liver and spleen.

4. Effects of Active Guided Biotic® (Control vs GB)

- GB reduced ($P<0.03$) *Salmonella* counts in the crop 7 days post-challenge (Figure 6).
- There was a weak trend for GB to reduce *Salmonella* in the crop ($P=0.40$) and caeca ($P=0.50$) on day 3, and the ileum ($P=0.58$) and caeca ($P=0.60$) on day 7.

TABLE 5: Example Bacteria

Optionally, the carrier cells are selected from this Table and/or the target cells are selected from this Table (eg, wherein the carrier and target cells are of a different species; or of the same species but are a different strain or the carrier cells are engineered but the target cells are wild-type or *vice versa*). For example the carrier cells are *E coli* cells and the target cells are *C dificile, E coli, Akkermansia, Enterobacteriacea, Ruminococcus, Faecalibacterium, Firmicutes, Bacteroidetes, Salmonella, Klebsiella, Pseudomonas, Acintenobacter* or *Streptococcus* cells.

Abiotrophia
Abiotrophia defectiva

Acaricomes
Acaricomes phytoseiuli

Acetitomaculum
Acetitomaculum ruminis

Acetivibrio
Acetivibrio cellulolyticus
Acetivibrio ethanolgignens
Acetivibrio multivorans

Acetoanaerobium
Acetoanaerobium noterae

Acidocella
Acidocella aminolytica
Acidocella facilis

Acidomonas
Acidomonas methanolica

Acidothermus
Acidothermus cellulolyticus

Acidovorax
Acidovorax anthurii
Acidovorax caeni
Acidovorax cattleyae
Acidovorax citrulli
Acidovorax defluvii
Acidovorax delafieldii

Actinomyces
Actinomyces bovis
Actinomyces denticolens
Actinomyces europaeus
Actinomyces georgiae
Actinomyces gerencseriae
Actinomyces hordeovulneris
Actinomyces howellii
Actinomyces hyovaginalis
Actinomyces israelii
Actinomyces johnsonii
Actinomyces meyeri
Actinomyces naeslundii
Actinomyces neuii
Actinomyces odontolyticus

Alkalilimnicola
Alkalilimnicola ehrlichii

Alkaliphilus
Alkaliphilus oremlandii
Alkaliphilus transvaalensis

Allochromatium
Allochromatium vinosum

Alloiococcus
Alloiococcus otitis

Allokutzneria
Allokutzneria albata

Aquaspirillum
Aquaspirillum polymorphum
Aquaspirillum putridiconchylium
Aquaspirillum serpens

Aquimarina
Aquimarina latercula

Arcanobacterium
Arcanobacterium haemolyticum
Arcanobacterium pyogenes

Archangium
Archangium gephyra

Acetobacter
Acetobacter aceti
Acetobacter cerevisiae
Acetobacter cibinongensis
Acetobacter estunensis
Acetobacter fabarum
Acetobacter ghanensis
Acetobacter indonesiensis
Acetobacter lovaniensis
Acetobacter malorum
Acetobacter nitrogenifigens
Acetobacter oeni
Acetobacter orientalis
Acetobacter orleanensis
Acetobacter pasteurianus
Acetobacter pornorurn
Acetobacter senegalensis
Acetobacter xylinus

Acetobacterium
Acetobacterium bakii

Acidovorax facilis
Acidovorax konjaci
Acidovorax temperans
Acidovorax valerianellae

Acinetobacter
Acinetobacter baumannii
Acinetobacter baylyi
Acinetobacter bouvetii
Acinetobacter calcoaceticus
Acinetobacter gerneri
Acinetobacter haemolyticus
Acinetobacter johnsonii
Acinetobacter junii
Acinetobacter lwoffi
Acinetobacter parvus
Acinetobacter radioresistens
Acinetobacter schindleri
Acinetobacter soli
Acinetobacter tandoii
Acinetobacter tjernbergiae

Actinomyces oris
Actinomyces radingae
Actinomyces slackii
Actinomyces turicensis
Actinomyces viscosus

Actinoplanes
Actinoplanes auranticolor
Actinoplanes brasiliensis
Actinoplanes consettensis
Actinoplanes deccanensis
Actinoplanes derwentensis
Actinoplanes digitatis
Actinoplanes durhamensis
Actinoplanes ferrugineus
Actinoplanes globisporus
Actinoplanes humidus
Actinoplanes italicus
Actinoplanes liguriensis
Actinoplanes lobatus
Actinoplanes missouriensis

Altererythrobacter
Altererythrobacter ishigakiensis

Altermonas
Altermonas haloplanktis
Altermonas macleodii

Alysiella
Alysiella crassa
Alysiella filiformis

Aminobacter
Aminobacter aganoensis
Aminobacter aminovorans
Aminobacter niigataensis

Aminobacterium
Aminobacterium mobile

Aminomonas
Aminomonas paucivorans

Arcobacter
Arcobacter butzleri
Arcobacter cryaerophilus
Arcobacter halophilus
Arcobacter nitrofigilis
Arcobacter skirrowii

Arhodomonas
Arhodomonas aquaeolei

Arsenophonus
Arsenophonus nasoniae

Arthrobacter
Arthrobacter agilis
Arthrobacter albus
Arthrobacter aurescens
Arthrobacter chlorophenolicus
Arthrobacter citreus

Acetobacterium carbinolicum
Acetobacterium dehalogenans
Acetobacterium fimetarium
Acetobacterium malicum
Acetobacterium paludosum
Acetobacterium tundrae
Acetobacterium wieringae
Acetobacterium woodii
Acetofilamentum
Acetofilamentum rigidum
Acetohalobium
Acetohalobium arabaticum
Acetomicrobium
Acetomicrobium faecale
Acetomicrobium flavidum
Acetonema
Acetonema longum
Acinetobacter towneri
Acinetobacter ursingii
Acinetobacter venetianus
Acrocarpospora
Acrocarpospora corrugata
Acrocarpospora macrocephala
Acrocarpospora pleiomorpha
Actibacter
Actibacter sediminis
Actinoalloteichus
Actinoalloteichus cyanogriseus
Actinoalloteichus hymeniacidonis
Actinoalloteichus spitiensis
Actinoplanes palleronii
Actinoplanes philippinensis
Actinoplanes rectilineatus
Actinoplanes regularis
Actinoplanes teichomyceticus
Actinoplanes utahensis
Actinopolyspora
Actinopolyspora halophila
Actinopolyspora mortivallis
Actinosynnema
Actinosynnema mirum
Actinotalea
Actinotalea fermentans
Aerococcus
Aerococcus sanguinicola
Aerococcus urinae
Ammoniphilus
Ammoniphilus oxalaticus
Ammoniphilus oxalivorans
Amphibacillus
Amphibacillus xylanus
Amphritea
Amphritea balenae
Amphritea japonica
Amycolatopsis
Amycolatopsis alba
Amycolatopsis albidoflavus
Amycolatopsis azurea
Amycolatopsis coloradensis
Amycolatopsis lurida
Amycolatopsis mediterranei
Amycolatopsis rifamycinica
Amycolatopsis rubida
Arthrobacter crystallopoietes
Arthrobacter cumminsii
Arthrobacter globiformis
Arthrobacter histidinolovorans
Arthrobacter ilicis
Arthrobacter luteus
Arthrobacter methylotrophus
Arthrobacter mysorens
Arthrobacter nicotianae
Arthrobacter nicotinovorans
Arthrobacter oxydans
Arthrobacter pascens
Arthrobacter phenanthrenivorans
Arthrobacter polychromogenes
Atrhrobacter protophormiae
Arthrobacter psychrolactophilus
Arthrobacter ramosus

Acetothermus

Acetothermus paucivorans

Acholeplasma

Acholeplasma axanthum

Acholeplasma brassicae

Acholeplasma cavigenitalium

Acholeplasma equifetale

Acholeplasma granularum

Acholeplasma hippikon

Acholeplasma laidlawii

Acholeplasma modicum

Acholeplasma morum

Acholeplasma multilocale

Acholeplasma oculi

Acholeplasma palmae

Acholeplasma parvum

Acholeplasma pleciae

Acholeplasma vituli

Actinobaccillus

Actinobacillus capsulatus

Actinobacillus delphinicola

Actinobacillus hominis

Actinobacillus indolicus

Actinobacillus lignieresii

Actinobacillus minor

Actinobacillus muris

Actinobacillus

pleuropneumoniae

Actinobacillus porcinus

Actinobacillus rossii

Actinobacillus scotiae

Actinobacillus seminis

Actinobacillus succinogenes

Actinobaccillus suis

Actinobacillus ureae

Actinobaculum

Actinobaculum massiliense

Actinobaculum schaalii

Aerococcus urinaeequi

Aerococcus urinaehominis

Aerococcus viridans

Aeromicrobium

Aeromicrobium erythreum

Aeromonas

Aeromonas

allosaccharophila

Aeromonas bestiarum

Aeromonas caviae

Aeromonas encheleia

Aeromonas

enteropelogenes

Aeromonas eucrenophila

Aeromonas ichthiosmia

Aeromonas jandaei

Aeromonas media

Aeromonas popoffii

Aeromonas sobria

Aeromonas veronii

Amycolatopsis sulphurea

Amycolatopsis tolypomycina

Anabaena

Anabaena cylindrica

Anabaena flos-aquae

Anabaena variabilis

Anaeroarcus

Anaeroarcus burkinensis

Anaerobaculum

Anaerobaculum mobile

Anaerobiospirillum

Anaerobiospirillum

succiniciproducens

Anaerobiospirillum thomasii

Anaerococcus

Anaerococcus hydrogenalis

Anaerococcus lactolyticus

Anaerococcus prevotii

Arthrobacter sulfonivorans

Arthrobacter sulfureus

Arthrobacter uratoxydans

Arthrobacter ureafaciens

Arthrobacter viscosus

Arthrobacter woluwensis

Asaia

Asaia bogorensis

Asanoa

Asanoa ferruginea

Asticcacaulis

Asticcacaulis biprosthecium

Asticcacaulis excentricus

Atopobacter

Atopobacter phocae

Atopobium

Atopobium fossor

Atopobium minutum

Actinobaculum suis

Achromobacter

Actinomyces urinale

Achromobacter denitrificans

Achromobacter insolitus

Achromobacter piechaudii

Achromobacter ruhlandii

Achromobacter spanius

Acidaminobacter

Acidaminobacter hydrogenoformans

Acidaminococcus

Acidaminococcus fermentans

Acidaminococcus intestini

Acidicaldus

Acidicaldus organivorans

Acidimicrobium

Acidimicrobium ferrooxidans

Agrobacterium

Agrobacterium

gelatinovorum

Actinocatenispora

Actinocatenispora rupis

Actinocatenispora thailandica

Actinocatenispora sera

Actinocorallia

Actinocorallia aurantiaca

Actinocorallia aurea

Actinocorallia cavernae

Actinocorallia glomerata

Actinocorallia herbida

Actinocorallia libanotica

Actinocorallia longicatena

Actinomadura

Actinomadura alba

Actinomadura atramentaria

Actinomadura

Agrococcus

Agrococcus citreus

Agrococcus jenensis

Agromonas

Agromonas oligotrophica

Agromyces

Agromyces fucosus

Agromyces hippuratus

Agromyces luteolus

Agromyces mediolanus

Agromyces ramosus

Agromyces rhizosphaerae

Akkermansia

Akkermansia muciniphila

Anaerococcus tetradius

Anaerococcus vaginalis

Anaerofustis

Anaerofustis stercorihominis

Anaeromusa

Anaeromusa acidaminophila

Anaeromyxobacter

Anaeromyxobacter dehalogenans

Anaerorhabdus

Anaerorhabdus furcosa

Anaerosinus

Anaerosinus glycerini

Anaerovirgula

Anaerovirgula multivorans

Atopobium parvulum

Atopobium rimae

Atopobium vaginae

Aureobacterium

Aureobacterium barkeri

Aurobacterium

Aurobacterium liquefaciens

Avibacterium

Avibacterium avium

Avibacterium gallinarum

Avibacterium paragallinarum

Avibacterium volantium

Azoarcus

Azoarcus indigens

Azoarcus tolulyticus

Azoarcus toluvorans

Acidiphilium
Acidiphilium acidophilum
Acidiphilium angustum
Acidiphilium cryptum
Acidiphilium multivorum
Acidiphilium organovorum
Acidiphilium rubrum

Acidisoma
Acidisoma sibiricum
Acidisoma tundrae

Acidisphaera
Acidisphaera rubrifaciens

Acidithiobacillus
Acidithiobacillus albertensis
Acidithiobacillus caldus
Acidithiobacillus ferrooxidans
Acidithiobacillus thiooxidans

bangladeshensis
Actinomadura catellatispora
Actinomadura chibensis
Actinomadura chokoriensis
Actinomadura citrea
Actinomadura coerulea
Actinomadura echinospora
Actinomadura fibrosa
Actinomadura formosensis
Actinomadura hibisca
Actinomadura kijaniata
Actinomadura latina
Actinomadura livida
Actinomadura luteofluorescens
Actinomadura macra
Actinomadura madurae
Actinomadura oligospora
Actinomadura pelletieri
Actinomadura rubrobrunea
Actinomadura rugatobispora

Albidiferax
Albidiferax ferrireducens

Albidovulum
Albidovulum inexpectatum

Alcaligenes
Alcaligenes denitrificans
Alcaligenes faecalis

Alcanivorax
Alcanivorax borkumensis
Alcanivorax jadensis

Algicola
Algicola bacteriolytica

Alicyclobacillus
Alicyclobacillus disulfidooxidans
Alicyclobacillus

Ancalomicrobium
Ancalomicrobium adetum

Ancylobacter
Ancylobacter aquaticus

Aneurinibacillus
Aneurinibacillus aneurinilyticus
Aneurinibacillus migulanus
Aneurinibacillus thermoaerophilus

Angiococcus
Angiococcus disciformis

Angulomicrobium
Angulomicrobium tetraedrale

Anoxybacillus
Anoxybacillus pushchinoensis

Azohydromonas
Azohydromonas australica
Azohydromonas lata

Azomonas
Azomonas agilis
Azomonas insignis
Azomonas macrocytogenes

Azorhizobium
Azorhizobium caulinodans

Azorhizophilus
Azorhizophilus paspali

Azospirillum
Azospirillum brasilense
Azospirillum halopraeferens
Azospirillum irakense

Azotobacter
Azotobacter beijerinckii

Acidobacterium
Acidobacterium capsulatum

Actinomadura umbrina
Actinomadura verrucosospora
Actinomadura vinacea
Actinomadura viridilutea
Actinomadura viridis
Actinomadura yumaensis
sendaiensis
Alicyclobacillus vulcanalis

Alishewanella
Alishewanella fetalis

Alkalibacillus
Alkalibacillus haloalkaliphilus

Aquabacterium
Aquabacterium commune
Aquabacterium parvum

Azotobacter chroococcum
Azotobacter nigricans
Azotobacter salinestris
Azotobacter vinelandii

Bacillus
[see below]

Bacteriovorax
Bacteriovorax stolpii

Bacteroides
Bacteroides caccae
Bacteroides coagulans
Bacteroides eggerthii
Bacteroides fragilis
Bacteroides galacturonicus
Bacteroides helcogenes
Bacteroides ovatus
Bacteroides pectinophilus
Bacteroides pyogenes
Bacteroides salyersiae

Bibersteinia
Bibersteinia trehalosi

Bifidobacterium
Bifidobacterium adolescentis
Bifidobacterium angulatum
Bifidobacterium animalis
Bifidobacterium asteroides
Bifidobacterium bifidum
Bifidobacterium boum
Bifidobacterium breve
Bifidobacterium catenulatum

Borrelia
Borrelia afzelii
Borrelia americana
Borrelia burgdorferi
Borrelia carolinensis
Borrelia coriaceae
Borrelia garinii
Borrelia japonica

Bosea
Bosea minatitlanensis
Bosea thiooxidans

Brevinema
Brevinema andersonii

Brevundimonas
Brevundimonas alba
Brevundimonas aurantiaca
Brevundimonas diminuta
Brevundimonas intermedia
Brevundimonas subvibrioides
Brevundimonas vancanneytii
Brevundimonas variabilis
Brevundimonas vesicularis

Bacteroides stercoris
Bacteroides suis
Bacteroides tectus
Bacteroides thetaiotaomicron
Bacteroides uniformis
Bacteroides ureolyticus
Bacteroides vulgatus

Balnearium
Balnearium lithotrophicum

Balneatrix
Balneatrix alpica

Balneola
Balneola vulgaris

Barnesiella
Barnesiella viscericola

Bartonella
Bartonella alsatica
Bartonella bacilliformis

Bifidobacterium choerinum
Bifidobacterium coryneforme
Bifidobacterium cuniculi
Bifidobacterium dentium
Bifidobacterium gallicum
Bifidobacterium gallinarum
Bifidobacterium indicum
Bifidobacterium longum
Bifidobacterium
magnumBifidobacterium
merycicum
Bifidobacterium minimum
Bifidobacterium
pseudocatenulatum
Bifidobacterium
pseudolongum
Bifidobacterium pullorum
Bifidobacterium ruminantium
Bifidobacterium saeculare
Bifidobacterium subtile

Brachybacterium
Brachybacterium
alimentarium
Brachybacterium faecium
Brachybacterium
paraconglomeratum
Brachybacterium rhamnosum
Brachybacterium
tyrofermentans

Brachyspira
Brachyspira alvinipulli
Brachyspira hyodysenteriae
Brachyspira innocens
Brachyspira murdochii
Brachyspira pilosicoli

Bradyrhizobium
Bradyrhizobium canariense

Brochothrix
Brochothrix campestris
Brochothrix thermosphacta

Brucella
Brucella canis
Brucella neotomae

Bryobacter
Bryobacter aggregatus

Burkholderia
Burkholderia ambifaria
Burkholderia andropogonis
Burkholderia anthina
Burkholderia caledonica
Burkholderia caryophylli
Burkholderia cenocepacia
Burkholderia cepacia
Burkholderia cocovenenans
Burkholderia dolosa

Bartonella clarridgeiae
Bartonella doshiae
Bartonella elizabethae
Bartonella grahamii
Bartonella henselae
Bartonella rochalimae
Bartonella vinsonii

Bavariicoccus
Bavariicoccus seileri

Bdellovibrio
Bdellovibrio bacteriovorus
Bdellovibrio exovorus

Beggiatoa
Beggiatoa alba

Beijerinckia
Beijerinckia derxii
Beijerinckia fluminensis

Bifidobacterium
thermophilum

Bilophila
Bilophila wadsworthia

Biostraticola
Biostraticola tofi

Bizionia
Bizionia argentinensis

Blastobacter
Blastobacter capsulatus
Blastobacter denitrificans

Blastococcus
Blastococcus aggregatus
Blastococcus saxobsidens

Blastochloris
Blastochloris viridis

Bradyrhizobium elkanii
Bradyrhizobium japonicum
Bradyrhizobium liaoningense

Brenneria
Brenneria alni
Brenneria nigrifluens
Brenneria quercina
Brenneria quercina
Brenneria salicis

Brevibacillus
Brevibacillus agri
Brevibacillus borstelensis
Brevibacillus brevis
Brevibacillus centrosporus
Brevibacillus choshinensis
Brevibacillus invocatus
Brevibacillus laterosporus
Brevibacillus parabrevis
Brevibacillus reuszeri

Burkholderia fungorum
Burkholderia glathei
Burkholderia glumae
Burkholderia graminis
Burkholderia kururiensis
Burkholderia multivorans
Burkholderia phenazinium
Burkholderia plantarii
Burkholderia pyrrocinia
Burkholderia silvatlantica
Burkholderia stabilis
Burkholderia thailandensis
Burkholderia tropica
Burkholderia unamae
Burkholderia vietnamiensis

Buttiauxella
Buttiauxella agrestis
Buttiauxella brennerae
Buttiauxella ferragutiae
Buttiauxella gaviniae

Beijerinckia indica

Beijerinckia mobilis

Belliella

Belliella baltica

Bellilinea

Bellilinea caldifistulae

Belnapia

Belnapia moabensis

Bergeriella

Bergeriella denitrificans

Beutenbergia

Beutenbergia cavernae

Blastomonas

Blastomonas natatoria

Blastopirellula

Blastopirellula marina

Blautia

Blautia coccoides

Blautia hansenii

Blautia producta

Blautia wexlerae

Bogoriella

Bogoriella caseilytica

Bordetella

Bordetella avium

Bordetella bronchiseptica

Bordetella hinzii

Bordetella holmesii

Bordetella parapertussis

Bordetella pertussis

Brevibacterium

Brevibacterium abidum

Brevibacterium album

Brevibacterium aurantiacum

Brevibacterium celere

Brevibacterium epidermidis

Brevibacterium frigoritolerans

Brevibacterium halotolerans

Brevibacterium iodinum

Brevibacterium linens

Brevibacterium lyticum

Brevibacterium mcbrellneri

Brevibacterium otitidis

Brevibacterium oxydans

Brevibacterium paucivorans

Brevibacterium stationis

Buttiauxella izardii

Buttiauxella noackiae

Buttiauxella warmboldiae

Butyrivibrio

Butyrivibrio fibrisolvens

Butyrivibrio hungatei

Butyrivibrio proteoclasticus

Bordetella petrii

Bordetella trematum

Bacillus

B. acidiceler
B. acidicola
B. acidiproducens
B. acidocaldarius
B. acidoterrestris
B. aeolius
B. aerius
B. aerophilus
B. agaradhaerens
B. agri
B. aidingensis
B. akibai
B. alcalophilus
B. algicola
B. alginolyticus
B. alkalidiazotrophicus
B. aminovorans
B. amylolyticus
B. andreesenii
B. aneurinilyticus
B. anthracis
B. aquimaris
B. arenosi
B. arseniciselenatis
B. arsenicus
B. aurantiacus
B. arvi
B. aryabhattai
B. asahii
B. atrophaeus
B. axarquiensis
B. azotofixans
B. glucanolyticus
B. gordonae
B. gottheilii
B. graminis
B. halmapalus
B. haloalkaliphilus
B. halochares
B. halodenitrificans
B. halodurans
B. halophilus
B. halosaccharovorans
B. hemicellulosilyticus
B. hemicentroti
B. herbersteinensis
B. horikoshii
B. horneckiae
B. taeanensis
B. tequilensis
B. thermantarcticus
B. thermoaerophilus
B. thermoamylovorans
B. thermocatenulatus
B. thermocloacae
B. thermocopriae
B. thermodenitrificans
B. thermoglucosidasius
B. thermolactis
B. thermoleovorans
B. thermophilus
B. thermoruber
B. thermosphaericus
B. thiaminolyticus
B. lautus
B. lehensis
B. lentimorbus
B. lentus
B. licheniformis
B. ligniniphilus
B. litoralis
B. locisalis
B. luciferensis
B. luteolus
B. luteus
B. macauensis
B. macerans
B. macquariensis
B. macyae
B. malacitensis

B. alkalinitrilicus
B. alkalisediminis
B. alkalitelluris
B. altitudinis
B. alveayuensis
B. alvei
B. amyloliquefaciens
- *B. a.* subsp. *amyloliquefaciens*
- *B. a.* subsp. *plantarum*

B. dipsosauri
B. drentensis
B. edaphicus
B. ehimensis
B. eiseniae
B. enclensis
B. endophyticus
B. endoradicis
B. farraginis
B. azotoformans
B. badius
B. barbaricus
B. bataviensis
B. beijingensis
B. benzoevorans
B. beringensis
B. berkeleyi
B. beveridgei
B. bogoriensis
B. boroniphilus
B. borstelensis
B. brevis Migula
B. butanolivorans
B. canaveralius
B. carboniphilus
B. cecembensis
B. cellulosilyticus
B. centrosporus
B. cereus
B. chagannorensis
B. horti
B. huizhouensis
B. humi
B. hwajinpoensis
B. idriensis
B. indicus
B. infantis
B. infernus
B. insolitus
B. invictae
B. iranensis
B. isabeliae
B. isronensis
B. jeotgali
B. kaustophilus
B. kobensis
B. kochii
B. kokeshiiformis
B. koreensis
B. korlensis
B. kribbensis
B. thioparans
B. thuringiensis
B. tiangshenii
B. trypoxylicola
B. tusciae
B. validus
B. vallismortis
B. vedderi
B. velezensis
B. vietnamensis
B. vireti
B. vulcani
B. wakoensis
B. weihenstephanensis
B. xiamenensis
B. xiaoxiensis
B. zhanjiangensis

B. peoriae
B. persepolensis
B. persicus
B. mannanilyticus
B. marisflavi
B. marismortui
B. marmarensis
B. massiliensis
B. megaterium
B. mesonae
B. methanolicus
B. methylotrophicus
B. migulanus
B. mojavensis
B. mucilaginosus
B. muralis
B. murimartini
B. mycoides
B. naganoensis
B. nanhaiensis
B. nanhaiisediminis
B. nealsonii
B. neidei
B. neizhouensis

B. fastidiosus
B. fengqiuensis
B. firmus
B. flexus
B. foraminis
B. fordii
B. formosus
B. fortis
B. fumarioli
B. funiculus
B. fusiformis
B. galactophilus
B. galactosidilyticus
B. galliciensis
B. gelatini
B. gibsonii
B. ginsengi
B. ginsengihumi
B. ginsengisoli
B. globisporus (eg, *B.*

B. chitinolyticus
B. chondroitinus
B. choshinensis
B. chungangensis
B. cibi
B. circulans
B. clarkii
B. clausii
B. coagulans
B. coahuilensis
B. cohnii
B. composti
B. curdlanolyticus
B. cycloheptanicus
B. cytotoxicus
B. daliensis
B. decisifrondis
B. decolorationis
B. deserti

B. krulwichiae
B. laevolacticus
B. larvae
B. laterosporus
B. salexigens
B. saliphilus
B. schlegelii
B. sediminis
B. selenatarsenatis
B. selenitireducens
B. seohaeanensis
B. shacheensis
B. shackletonii
B. siamensis
B. silvestris
B. simplex
B. siralis
B. smithii
B. soli
B. solimangrovi
B. solisalsi

B. pervagus
B. plakortidis
B. pocheonensis
B. polygoni
B. polymyxa
B. popilliae
B. pseudalcalophilus
B. pseudofirmus
B. pseudomycoides
B. psychrodurans
B. psychrophilus
B. psychrosaccharolyticus
B. psychrotolerans
B. puhvifaciens
B. pumilus
B. purgationiresistens
B. pycnus
B. qingdaonensis
B. qingshengii
B. reuszeri
B. rhizosphaerae

B. niabensis
B. niacini
B. novalis
B. oceanisediminis
B. odysseyi
B. okhensis
B. okuhidensis
B. oleronius
B. oryzaecorticis
B. oshimensis
B. pabuli
B. pakistanensis
B. pallidus
B. pallidus
B. panacisoli
B. panaciterrae
B. pantothenticus
B. parabrevis
B. paraflexus
B. pasteurii

g. subsp. *Globisporus;* or *B.*
g. subsp. *Marinus*)

B. songklensis
B. sonorensis
B. sphaericus
B. sporothermodurans
B. stearothermophilus
B. stratosphericus
B. subterraneus
B. subtilis (eg, *B.*
s. subsp. *Inaquosorum*; or *B.*
s. subsp. *Spizizeni*; or *B.*
s. subsp. *Subtilis*)

B. rigui
B. ruris
B. safensis
B. salarius

B. patagoniensis

Caenimonas
Caenimonas koreensis

Caldalkalibacillus
Caldalkalibacillus uzonensis

Caldanaerobacter
Caldanaerobacter subterraneus

Caldanaerobius
Caldanaerobius fijiensis

Campylobacter
Campylobacter coli
Campylobacter concisus
Campylobacter curvus
Campylobacter fetus
Campylobacter gracilis
Campylobacter helveticus
Campylobacter hominis
Campylobacter hyointestinalis
Campylobacter jejuni

Cardiobacterium
Cardiobacterium hominis

Carnimonas
Carnimonas nigrificans

Carnobacterium
Carnobacterium alterfunditum
Carnobacterium divergens

Catenuloplanes
Catenuloplanes atrovinosus
Catenuloplanes castaneus
Catenuloplanes crispus
Catenuloplanes indicus
Catenuloplanes japonicus
Catenuloplanes nepalensis
Catenuloplanes niger

Curtobacterium
Curtobacterium albidum
Curtobacterium citreus

Caldanaerobius
polysaccharolyticus
Caldanaerobius zeae

Caldanaerovirga
Caldanaerovirga acetigignens

Caldicellulosiruptor
Caldicellulosiruptor bescii
Caldicellulosiruptor kristjanssonii
Caldicellulosiruptor owensensis

Campylobacter lari
Campylobacter mucosalis
Campylobacter rectus
Campylobacter showae
Campylobacter sputorum
Campylobacter upsaliensis

Capnocytophaga
Capnocytophaga canimorsus
Capnocytophaga cynodegmi
Capnocytophaga gingivalis
Capnocytophaga granulosa
Capnocytophaga haemolytica
Capnocytophaga ochracea
Capnocytophaga sputigena

Carnobacterium funditum
Carnobacterium gallinarum
Carnobacterium
maltaromaticum
Carnobacterium mobile
Carnobacterium viridans

Caryophanon
Caryophanon latum
Caryophanon tenue

Catellatospora
Catellatospora citrea
Catellatospora
methionotrophica

Catenococcus
Catenococcus thiocycli

Chryseobacterium
Chryseobacterium
balustinum

Citrobacter
C. amalonaticus
C. braakii
C. diversus
C. farmeri
C. freundii
C. gillenii
C. koseri
C. murliniae
C. pasteurii[1]
C. rodentium
C. sedlakii
C. werkmanii
C. youngae

Clostridium
(see below)

Coccochloris

Coccochloris elabens

Corynebacterium

Corynebacterium flavescens

Corynebacterium variabile

Clostridium

Clostridium absonum, Clostridium aceticum, Clostridium acetireducens, Clostridium acetobutylicum, Clostridium acidisoli, Clostridium acididitolerans, Clostridium acidurici, Clostridium aerotolerans, Clostridium aestuarii, Clostridium akagii, Clostridium aldenense, Clostridium aldrichii, Clostridium algidicarni, Clostridium algidixylanolyticum, Clostridium algifaecis, Clostridium algoriphilum, Clostridium alkalicellulosi, Clostridium aminophilum, Clostridium aminovalericum, Clostridium amygdalinum, Clostridium amylolyticum, Clostridium arbusti, Clostridium arcticum, Clostridium argentinense, Clostridium asparagiforme, Clostridium aurantibutyricum, Clostridium autoethanogenum, Clostridium baratii, Clostridium barkeri, Clostridium bartlettii, Clostridium beijerinckii, Clostridium bifermentans, Clostridium bolteae, Clostridium bornimense, Clostridium botulinum, Clostridium bowmanii, Clostridium bryantii, Clostridium butyricum, Clostridium cadaveris, Clostridium caenicola, Clostridium caminithermale, Clostridium carboxidivorans, Clostridium carnis, Clostridium cavendishii, Clostridium celatum, Clostridium celerecrescens, Clostridium cellobioparum, Clostridium cellulofermentans, Clostridium cellulolyticum, Clostridium cellulosi, Clostridium cellulovorans, Clostridium chartatabidum, Clostridium chauvoei, Clostridium chromiireducens, Clostridium citroniae, Clostridium clariflavum, Clostridium clostridioforme, Clostridium coccoides, Clostridium cochlearium, Clostridium colletant, Clostridium colicanis, Clostridium colinum, Clostridium collagenovorans, Clostridium cylindrosporum, Clostridium difficile, Clostridium diolis, Clostridium disporicum, Clostridium drakei, Clostridium durum, Clostridium estertheticum, Clostridium estertheticum estertheticum, Clostridium estertheticum laramiense, Clostridium fallax, Clostridium felsineum, Clostridium fervidum, Clostridium fimetarium, Clostridium formicaceticum, Clostridium frigidicarnis, Clostridium

frigoris, Clostridium ganghwense, Clostridium gasigenes, Clostridium ghonii, Clostridium glycolicum, Clostridium glycyrrhizinilyticum, Clostridium grantii, Clostridium haemolyticum, Clostridium halophilum, Clostridium hastiforme, Clostridium hathewayi, Clostridium herbivorans, Clostridium hiranonis, Clostridium histolyticum, Clostridium homopropionicum, Clostridium huakuii, Clostridium hungatei, Clostridium hydrogeniformans, Clostridium hydroxybenzoicum, Clostridium hylemonae, Clostridium jejuense, Clostridium indolis, Clostridium innocuum, Clostridium intestinale, Clostridium irregulare, Clostridium isatidis, Clostridium josui, Clostridium kluyveri, Clostridium lactatifermentans, Clostridium lacusfryxellense, Clostridium laramiense, Clostridium lavalense, Clostridium lentocellum, Clostridium lentoputrescens, Clostridium leptum, Clostridium limosum, Clostridium litorale, Clostridium lituseburense, Clostridium ljungdahlii, Clostridium lortetii, Clostridium lundense, Clostridium magnum, Clostridium malenominatum, Clostridium mangenotii, Clostridium mayombei, Clostridium methoxybenzovorans, Clostridium methylpentosum, Clostridium neopropionicum, Clostridium nexile, Clostridium nitrophenolicum, Clostridium novyi, Clostridium oceanicum, Clostridium orbiscindens, Clostridium oroticum, Clostridium oxalicum, Clostridium papyrosolvens, Clostridium paradoxum, Clostridium paraperfringens (Alias: C. welchii), Clostridium paraputrificum, Clostridium pascui, Clostridium pasteurianum, Clostridium peptidivorans, Clostridium perenne, Clostridium perfringens, Clostridium pfennigii, Clostridium phytofermentans, Clostridium piliforme, Clostridium polysaccharolyticum, Clostridium populeti, Clostridium propionicum, Clostridium proteoclasticum, Clostridium proteolyticum, Clostridium psychrophilum, Clostridium puniceum, Clostridium purinilyticum, Clostridium putrefaciens, Clostridium putrificum, Clostridium quercicolum, Clostridium quinii, Clostridium ramosum, Clostridium rectum, Clostridium roseum, Clostridium saccharobutylicum, Clostridium saccharogumia, Clostridium saccharolyticum, Clostridium saccharoperbutylacetonicum, Clostridium sardiniense, Clostridium sartagoforme, Clostridium scatologenes, Clostridium schirmacherense, Clostridium scindens, Clostridium septicum, Clostridium sordellii, Clostridium sphenoides, Clostridium spiroforme, Clostridium sporogenes, Clostridium sporosphaeroides, Clostridium stercorarium, Clostridium stercorarium leptospartum, Clostridium stercorarium stercorarium, Clostridium stercorarium thermolacticum, Clostridium sticklandii, Clostridium straminisolvens, Clostridium subterminale, Clostridium sufflavum, Clostridium sulfidigenes, Clostridium symbiosum, Clostridium tagluense, Clostridium tepidiprofundi, Clostridium termitidis, Clostridium tertium, Clostridium tetani, Clostridium tetanomorphum, Clostridium thermaceticum, Clostridium thermautotrophicum, Clostridium thermoalcaliphilum, Clostridium thermobutyricum, Clostridium thermocellum, Clostridium thermocopriae, Clostridium thermohydrosulfuricum, Clostridium thermolacticum, Clostridium thermopalmarium, Clostridium

thermopapyrolyticum, Clostridium thermosaccharolyticum, Clostridium thermosuccinogenes, Clostridium thermosulfurigenes, Clostridium thiosulfatireducens, Clostridium tyrobutyricum, Clostridium uliginosum, Clostridium ultunense, Clostridium villosum, Clostridium vincentii, Clostridium viride, Clostridium xylanolyticum, Clostridium xylanovorans

Dactylosporangium
Dactylosporangium aurantiacum
Dactylosporangium fulvum
Dactylosporangium matsuzakiense
Dactylosporangium roseum
Dactylosporangium thailandense
Dactylosporangium vinaceum

Deinococcus
Deinococcus aerius
Deinococcus apachensis
Deinococcus aquaticus
Deinococcus aquatilis
Deinococcus caeni
Deinococcus radiodurans
Deinococcus radiophilus

Delftia
Delftia acidovorans

Desulfovibrio
Desulfovibrio desulfuricans

Diplococcus
Diplococcus pneumoniae

Echinicola
Echinicola pacifica
Echinicola vietnamensis

Enterobacter
E. aerogenes
E. amnigenus
E. agglomerans
E. arachidis
E. asburiae
E. cancerogenous
E. cloacae
E. cowanii
Enterobacter kobei
E. ludwigii
E. mori
E. nimipressuralis
E. oryzae
E. pulveris
E. pyrinus
E. radicincitans
E. taylorae

Faecalibacterium
Faecalibacterium prausnitzii

Fangia
Fangia hongkongensis

Fastidiosipila
Fastidiosipila sanguinis

Fusobacterium
Fusobacterium nucleatum

Flavobacterium
Flavobacterium antarcticum
Flavobacterium aquatile
Flavobacterium aquidurense
Flavobacterium balustinum
Flavobacterium croceum
Flavobacterium cucumis
Flavobacterium

E. dissolvens
E. gergoviae
E. helveticus
E. hormaechei
E. intermedius
E. turicensis
E. sakazakii Enterobacter soli

Enterococcus

Enterococcus durans
Enterococcus faecalis
Enterococcus faecium

Erwinia

Erwinia hapontici

Escherichia

Escherichia coli

daejeonense
Flavobacterium defluvii
Flavobacterium degerlachei
Flavobacterium denitrificans
Flavobacterium filum
Flavobacterium flevense
Flavobacterium frigidarium
Flavobacterium mizutaii
Flavobacterium okeanokoites

Gaetbulibacter

Gaetbulibacter saemankumensis

Gallibacterium

Gallibacterium anatis

Gallicola

Gallicola barnesae

Garciella

Garciella nitratireducens

Haemophilus

Haemophilus aegyptius
Haemophilus aphrophilus
Haemophilus felis
Haemophilus gallinarum
Haemophilus haemolyticus
Haemophilus influenzae
Haemophilus paracuniculus

Ideonella

Ideonella azotifigens

Idiomarina

Idiomarina abyssalis
Idiomarina baltica
Idiomarina fontislapidosi
Idiomarina loihiensis
Idiomarina ramblicola

Janibacter

Janibacter anophelis
Janibacter corallicola
Janibacter limosus
Janibacter melonis
Janibacter terrae

Jannaschia

Jannaschia cystaugens

Geobacillus
Geobacillus thermoglucosidasius
Geobacillus stearothermophilus
Geobacter
Geobacter bemidjiensis
Geobacter bremensis
Geobacter chapellei
Geobacter grbiciae
Geobacter hydrogenophilus
Geobacter lovleyi
Geobacter metallireducens
Geobacter pelophilus
Geobacter pickeringii
Geobacter sulfurreducens
Geodermatophilus
Geodermatophilus obscurus
Gluconacetobacter
Gluconacetobacter xylinus
Gordonia
Gordonia rubripertincta
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus
paraphrohaemolyticus
Haemophilus parasuis
Haemophilus pittmaniae
Hafnia
Hafnia alvei
Hahella
Hahella ganghwensis
Halalkalibacillus
Halalkalibacillus halophilus
Helicobacter
Helicobacter pylori
Idiomarina seosinensis
Idiomarina zobellii
Ignatzschineria
Ignatzschineria larvae
Ignavigranum
Ignavigranum ruoffiae
Ilumatobacter
Ilumatobacter fluminis
Ilyobacter
Ilyobacter delafieldii
Ilyobacter insuetus
Ilyobacter polytropus
Ilyobacter tartaricus
Jannaschia helgolandensis
Jannaschia pohangensis
Jannaschia rubra
Janthinobacterium
Janthinobacterium
agaricidamnosum
Janthinobacterium lividum
Jejuia
Jejuia pallidilutea
Jeotgalibacillus
Jeotgalibacillus
alimentarius
Jeotgalicoccus
Jeotgalicoccus halotolerans

Kaistia
Kaistia adipata
Kaistia soli
Kangiella
Kangiella aquimarina
Kangiella koreensis
Kerstersia
Kerstersia gyiorum
Kiloniella
Kiloniella laminariae
Klebsiella
K. granulomatis
K. oxytoca
K. pneumoniae
K. terrigena
K. varticola
Kluyvera
Kluyvera ascorbata

Labedella
Labedella gwakjiensis
Labrenzia
Labrenzia aggregata
Labrenzia alba
Labrenzia alexandrii
Labrenzia marina
Labrys
Labrys methylaminiphilus
Labrys miyagiensis
Labrys monachus
Labrys okinawensis
Labrys portucalensis
Lactobacillus
[see below]
Laceyella
Laceyella putida
Lechevalieria
Lechevalieria aerocolonigenes
Legionella

Listeria ivanovii
L. marthii
L. monocytogenes
L. newyorkensis
L. riparia
L. rocourtiae
L. seeligeri
L. weihenstephanensis
L. welshimeri
Listonella
Listonella anguillarum
Macrococcus
Macrococcus bovicus
Marinobacter
Marinobacter algicola
Marinobacter bryozoorum
Marinobacter flavimaris
Meiothermus
Meiothermus ruber

Micrococcus
Micrococcus luteus
Micrococcus lylae
Moraxella
Moraxella bovis
Moraxella nonliquefaciens
Moraxella osloensis
Nakamurella
Nakamurella multipartita
Nannocystis
Nannocystis pusilla
Natranaerobius
Natranaerobius
thermophilus
Natranaerobius trueperi
Naxibacter
Naxibacter alkalitolerans
Neisseria
Neisseria cinerea
Neisseria denitrificans
Neisseria gonorrhoeae

Nesterenkonia
Nesterenkonia holobia
Nocardia
Nocardia argentinensis
Nocardia corallina
Nocardia
otitidiscaviarum

Kocuria [see below]
Kocuria roasea
Kocuria varians
Kurthia
Kurthia zopfii

Listeria
L. aquatica
L. booriae
L. cornellensis
L. fleischmannii
L. floridensis
L. grandensis
L. grayi
L. innocua

Lactobacillus
L. acetotolerans
L. acidifarinae
L. acidipiscis
L. acidophilus
Lactobacillus agilis
L. algidus
L. alimentarius
L. amylolyticus
L. amylophilus
L. catenaformis
L. ceti
L. coleohominis
L. collinoides
L. composti
L. concavus
L. coryniformis
L. crispatus
L. crustorum

Methylophilus
Methylophilus methylotrophus

Microbacterium
Microbacterium ammoniaphilum
Microbacterium arborescens
Microbacterium liquefaciens
Microbacterium oxydans
L. mali
L. manihotivorans
L. mindensis
L. mucosae
L. murinus
L. nagelii
L. namurensis
L. nantensis
L. oligofermentans

Neisseria lactamica
Neisseria mucosa
Neisseria sicca
Neisseria subflava

Neptunomonas
Neptunomonas japonica
L. parakefiri
L. paralimentarius
L. paraplantarum
L. pentosus
L. perolens
L. plantarum
L. pontis
L. protectus
L. psittaci
L. sakei
L. salivarius
L. sanfranciscensis
L. satsumensis
L. secaliphilus
L. sharpeae
L. siliginis
L. spicheri
L. suebicus

L. amylotrophicus
L. amylovorus
L. animalis
L. antri
L. apodemi
L. aviarius
L. bifermentans
L. brevis
L. buchneri
L. camelliae
L. casei
L. kitasatonis
L. kunkeei
L. leichmannii
L. lindneri
L. malefermentans
L. curvatus
L. delbrueckii subsp.
bulgaricus
L. delbrueckii subsp.
delbrueckii
L. delbrueckii subsp. lactis
L. dextrinicus
L. diolivorans
L. equi
L. equigenerosi
L. farraginis
L. farciminis
L. fermentum
L. fornicalis
L. fructivorans
L. frumenti
L. oris
L. panis
L. pantheris
L. parabrevis
L. parabuchneri
L. paracasei
L. paracollinoides
L. parafarraginis
L. homohiochii
L. iners
L. ingluviei
L. intestinalis
L. fuchuensis
L. gallinarum
L. gasseri
L. rennini
L. reuteri
L. rhamnosus
L. rimae
L. rogosae
L. rossiae
L. ruminis
L. saerimneri
L. jensenii
L. johnsonii
L. kalixensis
L. keftiranofaciens
L. kefiri
L. kimchii
L. helveticus
L. hilgardii
L. thailandensis
L. ultunensis
L. vaccinostercus
L. vaginalis
L. versmoldensis
L. vini
L. vitulinus
L. zeae
L. zymae
L. gastricus
L. ghanensis
L. graminis
L. hammesii
L. hamsteri
L. harbinensis
L. hayakitensis

Legionella

Legionella adelaidensis
Legionella anisa
Legionella drancourtii
Legionella dresdenensis
Candidatus Legionella jeonii
Legionella jordanis
Legionella quinlivanii
Legionella rowbothamii

Legionella beliardensis
Legionella birminghamensis
Legionella bozemanae
Legionella brunensis
Legionella busanensis
Legionella cardiaca
Legionella cherrii
Legionella cincinnatiensis
Legionella clemsonensis
Legionella donaldsonii
Legionella drozanskii
Legionella dumoffii
Legionella erythra
Legionella fairfieldensis
Legionella fallonii
Legionella feeleii
Legionella geestiana
Legionella genomospecies
Legionella gormanii
Legionella gratiana
Legionella gresilensis
Legionella hackeliae
Legionella impletisoli
Legionella israelensis
Legionella jamestowniensis
Legionella lansingensis
Legionella londiniensis
Legionella longbeachae
Legionella lytica
Legionella maceachernii
Legionella massiliensis
Legionella micdadei
Legionella monrovica
Legionella moravica
Legionella nagasakiensis
Legionella nautarum
Legionella norrlandica
Legionella oakridgensis
Legionella parisiensis
Legionella pittsburghensis
Legionella pneumophila
Legionella quateirensis
Legionella rubrilucens
Legionella sainthelensi
Legionella santicrucis
Legionella shakespearei
Legionella spiritensis
Legionella steelei
Legionella steigerwaltii
Legionella taurinensis
Legionella tucsonensis
Legionella tunisiensis
Legionella wadsworthii
Legionella waltersii
Legionella worsleiensis
Legionella yabuuchiae

Oceanibulbus

Oceanibulbus indolifex

Paenibacillus

Paenibacillus thiaminolyticus

Prevotella

Prevotella albensis
Prevotella amnii

Quadrisphaera

Quadrisphaera granulorum

Oceanicaulis

Oceanicaulis alexandrii

Oceanicola

Oceanicola batsensis

Oceanicola granulosus

Oceanicola nanhaiensis

Oceanimonas

Oceanimonas baumannii

Oceaniserpentilla

Oceaniserpentilla haliotis

Oceanisphaera

Oceanisphaera donghaensis

Oceanisphaera litoralis

Oceanithermus

Oceanithermus desulfurans

Oceanithermus profundus

Oceanobacillus

Oceanobacillus caeni

Oceanospirillum

Oceanospirillum linum

Pantoea

Pantoea agglomerans

Paracoccus

Paracoccus alcaliphilus

Paucimonas

Paucimonas lemoignei

Pectobacterium

Pectobacterium aroideearum

Pectobacterium atrosepticum

Pectobacterium

betavasculorum

Pectobacterium cacticida

Pectobacterium carnegieana

Pectobacterium carotovorum

Pectobacterium chrysanthemi

Pectobacterium cypripedii

Pectobacterium rhapontici

Pectobacterium wasabiae

Planococcus

Planococcus citreus

Prevotella bergensis

Prevotella bivia

Prevotella brevis

Prevotella bryantii

Prevotella buccae

Prevotella buccalis

Prevotella copri

Prevotella dentalis

Prevotella denticola

Prevotella disiens

Prevotella histicola

Prevotella intermedia

Prevotella maculosa

Prevotella marshii

Prevotella melaninogenica

Prevotella micans

Prevotella multiformis

Prevotella nigrescens

Prevotella oralis

Prevotella oris

Prevotella oulorum

Quatrionicoccus

Quatrionicoccus

australiensis

Quinella

Quinella ovalis

Ralstonia

Ralstonia eutropha

Ralstonia insidiosa

Ralstonia mannitolilytica

Ralstonia pickettii

Ralstonia

pseudosolanacearum

Ralstonia syzygii

Ralstonia solanacearum

Ramlibacter

Ramlibacter henchirensis

Ramlibacter tataouinensis

Planomicrobium

Planomicrobium okeanokoites

Plesiomonas

Plesiomonas shigelloides

Proteus

Proteus vulgaris

Prevotella pallens

Prevotella salivae

Prevotella stercorea

Prevotella tannerae

Prevotella timonensis

Prevotella veroralis

Providencia

Providencia stuartii

Pseudomonas

Pseudomonas aeruginosa

Pseudomonas alcaligenes

Pseudomonas anguillispetica

Pseudomonas fluorescens

Pseudoalteromonas haloplanktis

Pseudomonas mendocina

Pseudomonas pseudoalcaligenes

Pseudomonas putida

Pseudomonas tutzeri

Pseudomonas syringae

Raoultella

Raoultella ornithinolytica

Raoultella planticola

Raoultella terrigena

Rathayibacter

Rathayibacter caricis

Rathayibacter festucae

Rathayibacter iranicus

Rathayibacter rathayi

Rathayibacter toxicus

Rathayibacter tritici

Rhodobacter

Rhodobacter sphaeroides

Ruegeria

Ruegeria gelatinovorans

Psychrobacter

Psychrobacter faecalis

Psychrobacter phenylpyruvicus

Saccharococcus

Saccharococcus thermophilus

Saccharomonospora

Saccharomonospora azurea

Saccharomonospora cyanea

Saccharomonospora viridis

Saccharophagus

Saccharophagus degradans

Saccharopolyspora

Saccharopolyspora erythraea

Saccharopolyspora gregorii

Saccharopolyspora hirsuta

Saccharopolyspora hordei

Sagittula

Sagittula stellata

Salegentibacter

Salegentibacter salegens

Salimicrobium

Salimicrobium album

Salinibacter

Salinibacter ruber

Salinicoccus

Salinicoccus alkaliphilus

Salinicoccus hispanicus

Salinicoccus roseus

Sanguibacter

Sanguibacter keddieii

Sanguibacter suarezii

Saprospira

Saprospira grandis

Sarcina

Sarcina maxima

Sarcina ventriculi

Sebaldella

Sebaldella termitidis

Stenotrophomonas

Stenotrophomonas maltophilia

Streptococcus

[also see below]

Streptomyces

Streptomyces achromogenes

Streptomyces cesalbus

Streptomyces cescaepitosus

Streptomyces cesdiastaticus

Streptomyces cesexfoliatus

Streptomyces fimbriatus

Streptomyces fradiae

Tatlockia

Tatlockia maceachernii

Tatlockia micdadei

Tenacibaculum

Tenacibaculum amylolyticum

Tenacibaculum discolor

Tenacibaculum gallaicum

Tenacibaculum lutimaris

Tenacibaculum mesophilum

Tenacibaculum skagerrakense

Saccharopolyspora rectivirgula
Saccharopolyspora spinosa
Saccharopolyspora taberi

Saccharothrix
Saccharothrix australiensis
Saccharothrix coeruleofusca
Saccharothrix espanaensis
Saccharothrix longispora
Saccharothrix mutabilis
Saccharothrix syringae
Saccharothrix tangerinus
Saccharothrix texasensis

Staphylococcus
S. arlettae
S. agnetis
S. aureus
S. auricularis
S. capitis
S. caprae
S. carnosus

Salinispora
Salinispora arenicola
Salinispora tropica

Salinivibrio
Salinivibrio costicola

Salmonella
Salmonella bongori
Salmonella enterica
Salmonella subterranea
Salmonella typhi

Serratia
Serratia fonticola
Serratia marcescens

Sphaerotilus
Sphaerotilus natans

Sphingobacterium
Sphingobacterium multivorum

Staphylococcus
[see below]

S. microti
S. muscae
S. nepalensis
S. pasteuri
S. petrasii
S. pettenkoferi
S. piscifermentans
S. equorum
S. felis
S. fleurettii
S. gallinarum
S. haemolyticus
S. hominis
S. schleiferi
S. sciuri
S. simiae
S. simulans
S. stepanovicii
S. succinus

Streptomyces fulvissimus
Streptomyces griseoruber
Streptomyces griseus
Streptomyces lavendulae

Streptomyces
phaeochromogenes
Streptomyces
thermodiastaticus

Streptomyces tubercidicus

Tepidanaerobacter
Tepidanaerobacter
syntrophicus

Tepidibacter
Tepidibacter
formicigenes
Tepidibacter
thalassicus

Thermus
Thermus aquaticus
Thermus filiformis
Thermus thermophilus

S. caseolyticus
S. chromogenes
S. cohnii
S. condimenti
S. delphini
S. devriesei
S. epidermidis

S. hyicus
S. intermedius
S. kloosii
S. leei
S. lentus
S. lugdunensis
S. lutrae
S. lyticans
S. massiliensis

S. pseudintermedius
S. pseudolugdunensis
S. pulvereri
S. rostri
S. saccharolyticus
S. saprophyticus

S. vitulinus
S. warneri
S. xylosus

Streptococcus

Streptococcus agalactiae
Streptococcus anginosus
Streptococcus bovis
Streptococcus canis
Streptococcus constellatus
Streptococcus downei
Streptococcus dysgalactiae
Streptococcus equines
Streptococcus faecalis
Streptococcus ferus

Streptococcus infantarius
Streptococcus iniae
Streptococcus intermedius
Streptococcus lactarius
Streptococcus milleri
Streptococcus mitis
Streptococcus mutans
Streptococcus oralis
Streptococcus tigurinus

Streptococcus orisratti
Streptococcus parasanguinis
Streptococcus peroris
Streptococcus pneumoniae
Streptococcus
pseudopneumoniae
Streptococcus pyogenes
Streptococcus ratti
Streptococcus salivariu

Streptococcus thermophilus
Streptococcus sanguinis
Streptococcus sobrinus
Streptococcus suis
Streptococcus uberis
Streptococcus vestibularis
Streptococcus viridans
Streptococcus
zooepidemicus

Uliginosibacterium

Uliginosibacterium gangwonense

Ulvibacter

Ulvibacter litoralis

Umezawaea

Umezawaea tangerina

Undibacterium

Undibacterium pigrum

Ureaplasma

Ureaplasma urealyticum

Ureibacillus

Ureibacillus composti
Ureibacillus suwonensis
Ureibacillus terrenus
Ureibacillus thermophilus
Ureibacillus thermosphaericus

Vagococcus

Vagococcus carniphilus
Vagococcus elongatus
Vagococcus fessus
Vagococcus fluvialis
Vagococcus lutrae
Vagococcus salmoninarum

Variovorax

Variovorax boronicumulans
Variovorax dokdonensis
Variovorax paradoxus
Variovorax soli

Veillonella

Veillonella atypica
Veillonella caviae
Veillonella criceti
Veillonella dispar
Veillonella montpellierensis

Vibrio

Vibrio aerogenes
Vibrio aestuarianus
Vibrio albensis
Vibrio alginolyticus
Vibrio campbellii
Vibrio cholerae
Vibrio cincinnatiensis
Vibrio coralliilyticus
Vibrio cyclitrophicus
Vibrio diazotrophicus
Vibrio fluvialis
Vibrio furnissii
Vibrio gazogenes
Vibrio halioticoli
Vibrio harveyi
Vibrio ichthyoenteri
Vibrio mediterranei
Vibrio metschnikovii

Virgibacillus

Virgibacillus halodenitrificans
Virgibacillus pantothenticus

Weissella

Weissella cibaria
Weissella confusa
Weissella halotolerans
Weissella hellenica
Weissella kandleri
Weissella koreensis
Weissella minor
Weissella paramesenteroides
Weissella soli
Weissella thailandensis
Weissella viridescens

Xanthobacter

Xanthobacter agilis
Xanthobacter aminoxidans
Xanthobacter autotrophicus
Xanthobacter flavus
Xanthobacter tagetidis
Xanthobacter viscosus

Xanthomonas

Xanthomonas albilineans
Xanthomonas alfalfae
Xanthomonas arboricola
Xanthomonas axonopodis
Xanthomonas campestris

Veillonella parvula
Veillonella ratti
Veillonella rodentium

Venenivibrio
Venenivibrio stagnispumantis

Verminephrobacter
Verminephrobacter eiseniae

Verrucomicrobium
Verrucomicrobium spinosum

Vibrio mytili
Vibrio natriegens
Vibrio navarrensis
Vibrio nereis
Vibrio nigripulchritudo
Vibrio ordalii
Vibrio orientalis
Vibrio parahaemolyticus
Vibrio pectenicida
Vibrio penaeicida
Vibrio proteolyticus
Vibrio shilonii
Vibrio splendidus
Vibrio tubiashii
Vibrio vulnificus

Williamsia
Williamsia marianensis
Williamsia maris
Williamsia serinedens

Winogradskyella
Winogradskyella thalassocola

Wolbachia
Wolbachia persica

Wolinella
Wolinella succinogenes

Zobellia
Zobellia galactanivorans
Zobellia uliginosa

Xanthomonas citri
Xanthomonas codiaei
Xanthomonas cucurbitae
Xanthomonas euvesicatoria
Xanthomonas fragariae
Xanthomonas fuscans
Xanthomonas gardneri
Xanthomonas hortorum
Xanthomonas hyacinthi
Xanthomonas perforans
Xanthomonas phaseoli
Xanthomonas pisi
Xanthomonas populi
Xanthomonas theicola
Xanthomonas translucens

Xanthomonas vesicatoria

Xenophilus

Xenophilus azovorans

Xenorhabdus

Xenorhabdus beddingii

Xenorhabdus bovienii

Xenorhabdus cabanillasii

Xenorhabdus doucetiae

Xenorhabdus griffiniae

Xenorhabdus hominickii

Xenorhabdus koppenhoeferi

Xenorhabdus nematophila

Xenorhabdus poinarii

Xylanibacter

Xylanibacter oryzae

Yangia

Yangia pacifica

Yaniella

Yaniella flava

Yaniella halotolerans

Yeosuana

Yeosuana aromativorans

Yersinia

Yersinia aldovae

Yersinia bercovieri

Yersinia enterocolitica

Yersinia entomophaga

Yersinia frederiksenii

Yersinia mollaretii

Yersinia philomiragia

Yersinia pestis

Yersinia pseudotuberculosis

Yersinia rohdei

Yersinia ruckeri

Yokenella

Yokenella regensburgei

Yonghaparkia

Yonghaparkia alkaliphila

Zavarzinia

Zavarzinia compransoris

Zoogloea

Zoogloea ramigera

Zoogloea resiniphila

Zooshikella

Zooshikella ganghwensis

Zunongwangia

Zunongwangia profunda

Zymobacter

Zymobacter palmae

Zymomonas

Zymomonas mobilis

Zymophilus

Zymophilus paucivorans

Zymophilus raffinosivorans

Xylella

Xylella fastidiosa

Xylophilus

Xylophilus ampelinus

Zobellella

Zobellella denitrificans

Zobellella taiwanensis

Zeaxanthinibacter

Zeaxanthinibacter enoshimensis

Zhihengliuella

Zhihengliuella halotolerans

Xylanibacterium

Xylanibacterium ulmi

Yersinia intermedia
Yersinia kristensenii

Table 6: Sequences:

SEQ ID NO:		
1	Salcr7-1 Sequence written 5' to 3'. Underlined: direct repeat Between direct repeats: spacer region corresponding (in 5' to 3' direction) to selected target sequences	
	TGAAACACGCATTGATTTGAGTCAGCTAGGAGGTGACTGAAGTATATTTTAGATGAAGATTATTTCTTAATAACTAAAAATATGGT ATAATACTCTTAATAAATGCAGTAATACAGGGGCTTTTCAAGACTGAAGTCTAGCTGAGACAAATAGTGCGATTACGAAATTTTTT AGACAAAAATAGTCTACGAGGTTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACCGCTTTGGGTATACGCATTTTGAAGTACGG GTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTGTCAACGGGTGTACTATATGTCTGTCATGGTTTTAGAGCTATGCTGTTTTT GAATGGTCCCAAAACGCAAACCAGTACCGAAGAGGCGCTCACGTTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTGCC GTTCTGGTCATCCTGCTCGAAGCCGCGTTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACCCAGAAATGAATCGGCCTGGCTTCA TTATCGGTTTTTAGAGCTATGCTGTTTTTGAATGGTCCCAAAACATCAGCAGGAAGCGCTCAAAAACATACTGCGTTTTTAGAGCTATG CTGTTTTTGAATGGTCCCAAAACAAGTGCGCCTGGTAGTCTTCCGGATAGCGGGTTTTTAGAGCTATGCTGTTTTTGAATGGACTCCAT TCTTCAGCACACTGAGACTTGTTGAGTTCCATGTTTTTAGAGCTATGCTGTTTTTGAATGGACTCCATTCAACATTGCCGATGATAACT TGAGAAAGAGGGTTAATACCAGCAGTCGGATACCTTCCTATTCTTTCTGTTAAAGCGTTTTCATGTTATAATAGGCAAAAGAAGAG TAGTGTGAT	
2	Salcr7-2 Sequence written 5' to 3'. Underlined: direct repeat Between direct repeats: spacer region corresponding (in 5' to 3' direction) to selected target sequences of *pipA*, *pipB*, *pipC*, *hilA*, *marT*, *sicP* and *sopB*.	
	TGAAACACGCATTGATTTGAGTCAGCTAGGAGGTGACTGAAGTATATTTTAGATGAAGATTATTTCTTAATAACTAAAAATATGGT ATAATACTCTTAATAAATGCAGTAATACAGGGGCTTTTCAAGACTGAAGTCTAGCTGAGACAAATAGTGCGATTACGAAATTTTTT AGACAAAAATAGTCTACGAGGTTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTCTTTTTTCATATGCGTAATTCATCAGTCTG GTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTATAACCGAGGATGGTTTTCTGAACCTGCGGTTTTAGAGCTATGCTGTTTT	

	GAATGGTCCCAAAACTATTAAATCGTTTATATGACGCGTTAGGCCGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACCACAA TAATCCACAAGCTTTAGGATTACTGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTCTATTTCCCTTCCAGCAGTCGCACC CCAGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTTATCGCCTGTTTGTGGCGATTCTATCTGGGTTTTAGAGCTATGCTG TTTTGAATGGTCCCAAAACTACTCACCGCGTCGAATATTTTCGGCAAAGGTTTTAGAGCTATGCTGTTTTGAATGGACTCCATTCTT CAGCACACTGAGACTTGTTGAGTTCCATGTTTTAGAGCTATGCTGTTTTGAATGGACTCCATTCAACATTGCCGATGATAACTTGA GAAAGAGGGTTAATACCAGCAGTCGGATACCTTCCTATTCTTTCTGTTAAAGCGTTTTCATGTTATAATAGGCAAAAGAAGAGTAG TGTGAT
3	Salcr7-3 Sequence written 5' to 3'. Underlined: direct repeat Between direct repeats: spacer region corresponding (in 5' to 3' direction) to selected target sequences of *pipA*, *pipB*, *pipC*, *hilA*, *marT*, *sicP* and *sopB*.
	TGAAACACGCATTGATTTGAGTCAGCTAGGAGGTGACTGAAGTATATTTTTAGATGAAGATTATTTCTTAATAACTAAAAATATGGT ATAATACTCTTAATAAATGCAGTAATACAGGGGCTTTTCAAGACTGAAGTCTAGCTGAGACAAATAGTGCGATTACGAAATTTTTT AGACAAAAATAGTCTACGAGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACCTATTTATTGAAGATGTAGACCATTCTGGG GTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACAGAAGCAATGAAAAGTGCAACTTCACCACGGTTTTAGAGCTATGCTGTTT TGAATGGTCCCAAAACATCGCTTGCCGCAAACCAGTACCGAAGAAGGTTTTTAGAGCTATGCTGTTTTTGAATGGTCCCAAAACTTCG ATTGACAGTACTATGGTTTTACTTACGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACATTGATTTGTCAGCAACCTTATAAA ACGCGGTTTTTAGAGCTATGCTGTTTTTGAATGGTCCCAAAACGAAGCAATCTTATAAAAGATATAATTCAAGGTTTTAGAGCTATGC TGTTTTTGAATGGTCCCAAAACTAACCTGGAGATTCAGAAACAAAATACGGGGTTTTTAGAGCTATGCTGTTTTTGAATGGACTCCATT CTTCAGCACACTGAGACTTGTTGAGTTCCATGTTTTTAGAGCTATGCTGTTTTTGAATGGACTCCATTCAACATTGCCGATGATAACTT GAGAAAGAGGGTTAATACCAGCAGTCGGATACCTTCCTATTCTTTCTGTTAAAGCGTTTTCATGTTATAATAGGCAAAAGAAGAGT AGTGTGAT
4	tracrRNA-Cas9 fragment sequence based on pCas9 Bold: tracrRNA Underlined: Cas9 coding sequence

CGAAATCATCCTGTGGAGCTTAGTAGGTTTAGCAAGATGGCAGCGCCTAAATGTAGAATGATAAAAGGATTAAGAGATTAATTTC
CCTAAAAATGATAAAACAAGCGTTTTGAAAGCGCTTGTTTTTTTGGTTTGCAGTCAGAGTAGAATAGAAGTATC**AAAAAAAGCAC**
CGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATGCTGTTTTGAATGGTTCCAACAAGA
TTATTTTATAACTTTTATAACAAATAATCAAGGAGAAAATTCAAAGAAATTTATCAGCCATAAAACAATACTTAATACTATAG
AATGATAACAAAATATAAACTACTTTTTAAAAGAATTTTGTGTTATAAATCTATTTATTAAGTATTGGGTAATATTTTTTTGAAGAGA
TATTTTGAAAAAGAAAAATTAAAGCATATTAAACTAATTTCGGAGGTCATTAAAACTATTATTGAAATCATCAAACTCATTATGGA
TTTAATTTAAACTTTTTTATTTTAGGAGGCAAAAAATGGATAAGAAATACTCAATAGGCTTAGATATCGGTCACAAATAGCGTCGGATG
GGCGGTGATCACTGATGAATATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACAGTATCAAAAAAAAT
CTTATAGGGGCTCTTTTATTTGACAGTGGAGAGACAGCGGAAGCGACTCGTCTCAAACGGACAGCTCGTAGAAGGTATACACGTC
GGAAGAATCGTATTTGTTATCTACAGGAGATTTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAG
TCTTTTTTGGTGGAAGAAGCAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGATGAAGTTGCTTATCATGAGAAATA
TCCAACTATCTATCATCTGCGAAAAAAAATTGGTAGATTCTACTGATAAAGCGGATTTGCGCTTAATCTATTTGGCCTTAGCGCATA
TGATTAAGTTTCGTGGTCATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACAAACTATTTATCCAGTTGGTAC
AAACCTACAATCAATTATTTGAAGAAAACCCTATTAACGCAAGTGGAGTAGATGCTAAAGCGATTCTTTCTTCTGCACGATTGAGTAA
ATCAAGACGATTAGAAAAATCTCATTGCTCAGCTCCCCGGTGAGAAGAAAAATGGCTTATTTGGGAAATCTCATTGCTTTGTCATTGG
GTTTGACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGATACTTACGATGATTTA
GATAATTTATTGGCGCAAATTGGAGATCAATATGCTGATTTGTTTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAGAT
ATCCTAAGAGTAAATACTGAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAACGCTACGATGAACATCATCAAGACTTGA
CTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAAGTATAAAGAAATCTTTTTTGATCAATCAAAAAAACGGATATGCAGGT
TATATTGATGGGGGAGCTAGCCAAGAAGAATTTTATAAATTTATCAAACCAATTTTAGAAAAAAATGGATGTACTGAGGAATTAT
TGGTGAAACTAAATCGTGAAGATTTTGCTGCGCAAGCAACGGACCTTTGACAACGCTCTATTCCCCATCAAATTCACTTGGGTGAG
CTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAAAAGACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCG
AATTCCTTATTATGTTGGTCCATTGGCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGGTCTGAAGAAACAATTACCCCAT
GGAATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGCATGACAAACTTTGATAAAAATCTTCCAAAT
GAAAAAGTACTACCAAAACATAGTTTGCTTTATGAGTATTTTACGGTTTATAACGAATTGACAAAGGTCAAATATGTTACTGAAGG
AATGCGAAAAACCAGCATTTCTTTCAGGTGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAACAAATCGAAAAGTAACCGTT
AAGCAATTAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGAAATTTCAGGAGTTGAAGATAGATTTAATGCTT
CATTAGGTACCTACCATGATTTGCTAAAAATTATTAAAGATAAAGATTTTTTTGGATAATGAAGAAAATGAAGATATCTTAGAGGA

	TATTGTTTTAACATTGACCTTATTTGAAGATAGGGAGATGATTGAGGAAAGACTTAAAACATATGCTCACCTCTTTGATGATAAGG
	TGATGAAACAGCTTAAACGTCGCCGTTATACTGGTTGGGACGTTTGTCTCGAAAATTGATTAATGGTATTAGGGATAAGCAATCT
	GGCAAAACAATATTAGATTTTTTGAAATCAGATGGTTTTGCCAATCGCAATTTTATGCAGCTGATCCATGATGATAGTTTGACATT
	TAAAGAAGACATTCAAAAAGCACAAGTGTCTGGACAAGGCGATAGTTTACATGAACATATTGCAAATTTAGCTGGTAGCCCTGCT
	ATTAAAAAAGGTATTTTACAGACTGTAAAAGTTGTTGATGAATTGGTCAAAGTAATGGGGCGGCATAAGCCAGAAAATATCGTTA
	TTGAAATGGCACGTGAAAATCAGACAACTCAAAAGGGCCAGAAAAAATTCGCGAGAGCGTATGAAACGAATCGAAGAAGGTATCA
	AAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAATACTCAATTGCAAAATGAAAAGCTCTATCTCTATTATCTCCAA
	AATGGAAGAGACATGTATGTGGACCAAGAATTAGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGTTT
	CCTTAAAGACGATTCAATAGACAATAAGGTCTTAACGCGTTCTGATAAAAATCGTGGTAAATCGGATAACGTTCCAAGTGAAGAA
	GTAGTCAAAAAAGATGAAAAAACTATTGGAGACAACTTCTAAACGCCAAGTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAG
	CTGAACGTGGAGGTTTGAGTGAACTTGATTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATGT
	GGCACAAATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTTATTCGAGAGGTTAAAGTGATTACCTTAAAA
	TCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAATTCTATAAAGTACGTGAGATTAACAATTACCATGCCCATGATGCGTA
	TCTAAATGCCGTCGTTGGAACTGCTTTGATTAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAAAGTTTTATG
	ATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCGCAAAATATTTCTTTTACTCTAATATCATGAACTTC
	TTCAAAACAGAAATTACACTTGCAAATGGAGAGATTCGCAAACGCCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGTCT
	GGGATAAAGGGCGAGATTTTGCCACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCAAGAAAACAGAAGTACAGAC
	AGGCGGATTCTCCAAGGAGTCAATTTTACCAAAAAGAAATTCGGACAAGCTTATTGCTCGTAAAAAAGACTGGGATCCAAAAAAA
	TATGGTGGTTTTGATAGTCCAACGGTAGCTTATTCAGTCCTAGTGGTTGCTAAGGTGGAAAAAGGGAAATCGAAGAAGTTTAAAAT
	CCGTTAAAGAGTTACTAGGGATCACAATTATGGAAAGAAGTTCCTTTGAAAAAAATCCGATTGACTTTTTAGAAGCTAAAGGATA
	TAAGGAAGTTAAAAAAGACTTAATCATTAAACTACCTAAATATAGTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGCTGGCT
	AGTGCCGGAGAATTACAAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGTCATTATGAAA
	AGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTTGTGGAGCAGCATTATTTAGATGAGATTATTGAGCA
	AATCAGTGAATTTTCTAAGCGTGTTATTTTAGCAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAACAAACATAGAGACAAAAC
	CAATACGTGAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGCTTTTTAAATATTTTGATACA
	ACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAGATGCCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAAC
	ACGCATTGATTTGAGTCAGCTAGGAGGTGACTGA
5	Salcr3.2 (crRNA-pipC-hilA-marT)

	Sequence written 5' to 3'. Underlined: direct repeat Between direct repeats: spacer region corresponding (in 5' to 3' direction) to selected target sequences of *pipC-hilA-marT*
	TGTCGACGGTATATATTTTAGATGAAGATTATTTCTTAATAACTAAAAAATATGGTATAAATACTCTTAATAAATGCAGTAATACAGGGG CTTTTCAAGACTGAAGTCTAGCTGAGACAAATAGTGCGATTACGAAATTTTTTAGACAAAAATAGTCTACGAGGTTTTAGAGCTAT GCTGTTTTGAATGGTCCCAAAACATCGCTTGCCGCAAACCAGTACCGAAGAAGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAA AACTTCGATTGACAGTACTATGGTTTACTTACGGTTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACATTGATTTGTCAGCAACC TTATAAAACGCGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTTCAGCACACTGAGAACTTGTTGAGTTCCATGTTTTAGA GCTATGCTGTTTTGAATGGTCTCCATTCAACATTGCCGATGATAACTTGAGAAAGAGGGTTAATACCAGCAGTCGGATACCTTCCT ATTCTTTCTGTTAAAGCGTTTTCATGTTATAATAGGCAAAAGAAGAGTAGTGTGAT
6	mob (oriT)
	TTGAGCACCGCCAGGTGCGAATAAGGGACAGTGAAGAAGGAACACCCGCTCGCGGGTGGGCCTACTTCACCTATCCTGCCCGGCT GACGCCGTTGGATACACCAAGGAAAGTCTACACGAA
7	pZA31MCS
	ATCCCATGGTACGCGTGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTC GGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCTAGGGATATATTCCGCTTCCTCGCTCACTGACTCGCTACGCTC GGTCGTTCGACTGCGGCGAGCGGGAAATGGCTTACGAACGGGGCGGAGATTTCCTGGAAGATGCCAGGAAGATACTTAACAGGGA AGTGAGAGGGCCGCGGCAAAGCCGTTTTTCCATAGGCTCCGCCCCCCTGACAAGCATCACGAAATCTGACGCTCAAATCAGTGGT GGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGCGGCTCCCTCGTGCGCTCTCCTGTTCCTGCCTTTCGGTTT ACCGGTGTCATTCCGCTGTTATGGCCGCGTTTGTCTCATTCCACGCCTGACACTCAGTTCCGGGTAGGCAGTTCGCTCCAAGCTGG ACTGTATGCACGAACCCCCCGTTCAGTCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGAAAGACATGCA AAAGCACCACTGGCAGCAGCCACTGGTAATTGATTTAGAGGAGTTAGTCTTGAAGTCATGCGCCGGTTAAGGCTAAACTGAAAGG ACAAGTTTTGGTGACTGCGCTCCTCCAAGCCAGTTACCTCGGTTCAAAGAGTTGGTAGCTCAGAGAACCTTCGAAAAACCGCCCTG CAAGGCGGTTTTTTCGTTTTCAGAGCAAGAGATTACGCGCAGACCAAAACGATCTCAAGAAGATCATCTTATTAATCAGATAAAA TATTACTAGATTTCAGTGCAATTTATCTCTTCAAATGTAGCACCTGAAGTCAGCCCCATACGATATAAGTTGTTACTAGTGCTTGGA TTCTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCA ACAGGAGTCCAAGCGAGCTCGATATCAAATTACGCCCCGCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCC GACATGGAAGCCATCACAGACGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATATTTGCCC ATGGTGAAAACGGGGGCGAAGAAGTTGTCCATATTGGCCACGTTTAAATCAAAACTGGTGAAACTCACCCAGGGATTGGCTGAGA CGAAAAACATATTCTCAATAAACCCTTTAGGGAAATAGGCCAGGTTTTCACCGTAACACGCCACATCTTGCGAATATATGTGTAG

	AAACTGCCGGAAATCGTCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGGAAAACGGTGTAACAAGGGTGA ACACTATCCCATATCACCAGCTCACCGTCTTTCATTGCCATACGAAATTCCGGATGAGCATTCATCAGGCGGGCAAGAATGTGAAT AAAGGCCGGATAAAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTAC ATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCT CCATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTCATTATGGTGAAAGTTG GAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAGATATCGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAA AATAGGCGTATCACGAGGCCCTTTCGTCTTCAC
8	spCas9-6 forward primer
	ATTGTTTGTGGAGCAGCATAAGC
9	mob2 reverse primer
	GCCTCTAGCACGCGTACCATGGGAT
10	trfA module for genomic insertion DH10B (written 5' to 3') Bracketed sequence: encodes trfA mRNA (complement strand) Capital letters at 5': bi-directional synthetic rho-independent terminator to prevent read-through from the trfA gene as well as upstream gene on the chromosome Lower case letters at 3': J23105 promoter, a weak to intermediate strength promoter (capital T indicates transcription start site)
	CTTAATAAAAAACCCGCTTGGGCAAGCGGGTTTTATTGTGCAATTAGGCC[CTAGCGTTTGCAATGCACCAGGTCATCATTGACCCA GGCGTGTTCCACCAGGCCGCTGCCTCGCAACTCTTCGCAGGCTTCGCCGACCTGCTCGCGCCACTTCTTCACGCGGGTGGAATCCG ATCCGCACATGAGGCGGAAGGTTTCCAGCTTGAGCGGGTACGGCTCCCGGTGCGAGCTGAAATAGTCGAACATCCGTCGGGCCGT CGGCGACAGCTTGCGGTACTTCTCCCATATGAATTTCGTCTAGTGGTCGCCAGCAAACAGCACGACGATTTCCTCGTCGATCAGGA CCTGGCAACGGGACGTTTTCTTGCCACGGTCCAGGACGCGGAAGCGGTGCAGCAGCGACACCGATTCCAGGTGCCCAACGCGGTC GGACGTGAAGTCCATCGCCGTCGCCTGTAGGCGCGACAGGCATTCCTCGGCCTTCGTGTAATACCGGCCATTGATCGACCAGCCC AGGTCCTGGCAAAGCTCGTAGAACGTGAAGGTGATCGGCTCGCCGATAGGGGTGCGCTTCGCGTACTCCAACACCTGCTGCCACA CCAGTTCGTCATCGTCGGCCCGCAGCTCGACGCCGGTGTAGGTGATCTTCACGTCCTTGTTGACGTGGAAAATGACCTTGTTTTGC

	AGCGCCTCCGCGCGGGATTTTCTTGTTGCGCGTGGTGAACAGGGCAGAGCGGGCCGTGTCGTTTGGCATCGCTCGCATCGTGTCCGG
	CCACGGCGCAATATCGAACAAGGAAAGCTGCATTTCCTTGATCTGCTTCGTGTGTTTCAGCAACGCGGCCTGCTTGGCCTCGC
	TGACCTGTTTTGCCAGGTCCTCGCCGGCGGTTTTTCGCTTCTTGGTCGTCATAGTTCCTCGCGTGTCGATCATCGACTTCGCCA
	AACCTGCCGCCTCCTGTTCGAGACGACGAACGCTCCACGGCGGCCGATGGCGCGGGCAGGGCAGGGGAGCCAGTTGCACGC
	TGTCGCGCTCGATCTTGGCCGTAGCTTGCTGGACCATCGAGCCGACGGACTGGAAGGTTTCGCGGGGCGCACGCATGACGGTGCG
	GCTTGCGATGGTTTCGGCATCCTCGGCGGAAAACCCCGCGTCGATCAGTTCTTGCCTGTATGCCTTCCGGTCAAACGTCCGATTCA
	TTCACCCTCCTTGCGGGATTGCCCCGACTCACGCCGGGGCAATGTGCCCTTgTagctagcatagtacctaggactgagctagccgtaaa
11	*aroA* coding sequence
	ATGGAATCCCTGACGTTACAACCCATCGCTCGTGTCGATGGCACTATTAATCTGCCCGGTTCCAAGAGCGTTTCTAACCGCGCTTT
	ATTGCTGGCGGCATTAGCACACGGCAAAACAGTATTAACCAATCTGCTGGATAGCGATGACGTGCGCCATATGCTGAATGCATTA
	ACAGCGTTAGGGGTAAGCTATACGCTTTCAGCCGATCGTTACGCGTTGCGAAATTATCGGTAACGGCGGTCCATTACACGCAGAAG
	GTGCCCTGGAGTTGTTCCTCGGTAACGCCGGAACGGCAATGCGTCCGCTGGCGGCAGCTCTCTTGTCTGGGTAGCAATGATATTGTG
	CTGACCGGTGAGCCGCGTATGAAAGAACGCCCGATTGGTCATCTGGTGGATGCGCTGCGCCTGGGCGGGGCGAAGATCACTTACC
	TGGAACAAGAAAATTATCCGCCGTTGCGTTTACAGGGCGGCTTTACTGGCGGCAACGTTGACGTTGATGGCTCCGTTTCCAGCCAA
	TTCCTCACCGCACTGTTAATGACTGCGCCTCTTGCGCCGGAAGATACGGTGATTCGTATTAAAGGCGATCTGGTTTCTAAACCTTA
	TATCGACATCACACTCAATCTGATGAAGACGTTTGGTGTTGAAATTGAAAATCAGCACTATCAACAATTTGTCGTAAAAGGCGGG
	CAGTCTTATCAGTCTCCGGGTACTTATTTGGTCGAAGGCGATGCATCTTCGGCTTCTTACTTTCTGGCAGCAGCAGCAATCAAAGG
	CGGCACTGTAAAAGTGACCGGTATTGGACGTAACAGTATGCAGGGTGATATTCGCTTTGCTGATGTGCTGGAAAAAATGGGCGCG
	ACCATTTGCTGGGGCGATGATTATATTTCCTGCACGCGTGGTGAACTGAACGCTATTGATATGGATATGAACCATATTCCTGATGC
	GGCGATGACCATTGCCACGGCGGCGTTATTTGCAAAAGGCACCACCACGCTGCGCAATATCTATAACTGGCGTGTTAAAGAGACC
	GATCGCCTGTTTGCGATGGCAACAGAACTGCGTAAAGTCGGCGCGGAAGTGGAAGAGGGGCACGATTACATTCGTATCACTCCTC

	CGGAAAAAACTGAACTTTGCCGAGATCGCGACATACAATGATCACCGGATGGCGATGTGTTTCTCGCTGGTGGCGTTGTCAGATAC ACCAGTGACGATTCTTGATCCCAAATGCACGGCCAAAACATTTCCGGATTATTTCGAGCAGCTGGCGCGGATTAGCCAGGCAGCC TGA
12	oriV RK2
	Tgacacttgaggggcgtttagagcgagccaggaaagccgacccccctccttggagtaaaaaccctgcggcgttgcagccggcacggatcttccgatcgggcgcggtggtggccgcgtctgtgacctaaaaaggg gggagtccagaggggcgcagcccctttgggcatagcgcagcgtaatcggagacgtaatttgagcatttccaggcgcttgcgcctggtcaacgaaagagtcagcgccgtagggctgccatttttgggtgaggccg ttcgcggccgaggggcgcagcccctgggggatgtggaggcccgcgttagcgggccgggaggttcgagaaggggggggcacccccccttcggcgtgcgcggtcacgcgccagggcgcagccctggttaaaaa caaggtttataaatattggtttaaaagcaggttaaagcaggtggccgaaaaacgggcggaaaaccttgcaaatgctggattttctgcctgtggacagcccctcaaatgtcaatagtgcgcccctcatctg tcatcactctgccctcaagtgtcaaggatcgcgcccctcatctgtcagtagtcgcgccctcaagtgtcaataccgcagggcacttatccccagggttgtccacatcatctgtgggaaactcgcgtaaaatcaggcgtt ttcgccgatttgcgaggctggccagctccacgtcgccggccgaaatcgagcctgcccctcatctgtcaacgccgccgcgcgatttgtcaacgtccgcccctcatctgtcagtgagggccaag ttttccgctggtgatccacaacgccggcggccgcggtgtctcgcacacggcttcgacggcgtttctggcgcgtttgcagggccatagacggccgccagcccagccgcgaggcaaccagccccggtgagcgtcgg aaaggcgctggaagccccgtagcgacgcggagaggggcgagacaagccaagggcgcaggctcgatgcgcagcacgacatagccggttctcgcaaggacgagaatttccctgcggtgcccctcaagtgtcaa
13	tra2 module trbB-L RK2
	ttgcaggctaaacactttcggtatatcgtttgcctgtgcgataaatgttgctaattgatttgttgcgtagggggttactgaaaagtgagcgggaaaagaagagtttcagaccatcaaggagcgggccaagcgcaagctggaac gcgacatgggtgcggacctgttggccgcgctcaacgacccgaaaaccgttgaagtcatgctcaacggcaaggtgtggcacgaacgccttggcgagccgatgcggtacatctgcgacatgcggcccag ccagtcgcaggcgattatagaaacggtggccggattccacggcaaagaggtcacgcggcattcgccccatcctggaaggcgagttcccctggatggcagccgcttgccgggccaattgccgccggtcgtggccgc gccaacctttgcgatccgcaagcgcgcggtcgccatcttcacgctggaacagtacgtcgaggcgggcatcatgacccgcgagcaatacgaggtcattaaaagcgccgtcgcggcgcatcgaaacatcctcgtcatt ggcggtactggctcgggcaagaccacgctcgtcaacgcgatcatcaatgaaatggtcgccttcaacccgtctgagcgcgtcgtcatcatcgaggacaccggcgaaaatccagtgcgccgcagagaacgccgtccaa taccacaccagcatcgacgtctcgatgacgctgctgctcaagacaacgctgcgtatgcgccccgaccgcatcctggtcgtgaggtacgtggccccgaagcccttgatctgttgatggcctggaacaccgggcatg aaggaggtgccgcccaccctgcacgcaaacaacccccaaagcgggcctgagccggctgccatgcttatcagcatgcacccggattcaccgaaacccattgagccgctgattggcgaggcggttcatgtggtcgtcc

atatcgccaggaccccctagcggccgtcgagtgcaagaaattctcgaagttcttggttacgagaacggccagtacatcaccaaaaaccctgtaaggagtatttccaatgacaacggctgttccgttccgtctgaccatgaa
tcgcgggcatttttgttctaccttgccgtgttcttcgttatccgcgcatccggcgatggcctcggaaggcaccgggcggcagcttgccatatgagagctggctgacgaacctgcgcaactccgtaaccgg
gcccggtggcctgcgctgtccatcatcggcatcgtcgtcgccggcggcgtgctgatcttccgggctgcaactcaatgccttcctgttctggtcgctgctggtcgtcggcgcaga
acgtgatgagcaccttcttcggtctgtggtgccgaaatcgcggcccctcggcaacgggcgcctgcaccaggtgcaagtcgcggcggcgcgatgccgtgctgcgtgcggtagcggctcgccttaatcatggctc
tgcgcacgatcccccatccgtcgcgcaggcaaccgagaaaacctgttcatgggtggtgatcgtgaactggtgatgttctcgggcctgatggcgtttgcgctgatttttcagcgcccaagagctgcgggccaccgtggtc
ggtctgatcctgtggttcgggggcgctctatgcgttccgtgtacctgcgtcaccgccggtacaagccgtattacccggcccgctcgaccccgttccgcgagaa
caccaatagccaaggaaggaagcaatccgatgatccaagcaatttgcgattgcaatcgcgggcctcggcgcgcttctgttgttcatcctcttttgcccgcatccgcgcggtcgatgccgaactgaaactgaaaaagcatcgt
tccaaggacgccggcctggccgatctgctcaactacgccgctgtcgtcgatgacggcgtaatcgtgggcaagaacggcagctttatggctgcctggctgtacaaggggcgatgacaacgcaagcaccgacca
gcagcgcgaagtagtgttcgcccgcatcaaccaggccctcgcgggcctgggaagtgggtggatgatccatgtggacgccgtgcggtcctgctccgaactacgcggagcgggggcctgtcggcgttccctgac
cgtctgacggcgagcgattgaagaagagcgccggcggcatttcgagaggcctgggaacgatgtacgagggctatttcgtcctcacctgattcccgccgctgcccagcgcaagttcgtcgagctgatgttt
gacgacgacgcgaccgcacggatcgcaaggcgcgcacgcgggggcctcatcgaccaatttcaagcgtgacgtgcgcagcatcgagtcgcgctgtcgtcggccgtgtcgctcactcgcttgaagggcacaaga
tcgtcaacgaggacggcacgtcacgcatgacgacttcctgcgctggctgcaattctgcgtgacgggcctgcaccatccggtgcaggccctggtcggcggacagg
aaatgtgggcgcgggtagtgtgccccaagtcgtccagtggtcgctctcgaaggcttcccctggaagtcctgcccggcatcctgacgcgctgccgagtatcggtggtcgag
ccggttcatcttcatggaccagcacgaagccgtgaagcacctcgacaagttccgcaagaagatggcgcggcttcttcgaccaggtgttcaacacgaacaccgggcccggtcgatcaggacgcgcctt
tcgatggtggccgatgctgaggcggcccattgccgaagtcaacagcggcatcgtggccgtgggctactacaccagcgtcgtgctgatggatgaggaccgcacgcgcctggcccgcgatgttga
aaaggccgtcaaccggttggcttttgccgcgcgcatcgagtccatcaacacccctggacgccttccttggtagtttgccgggccacggcgtggaaaacgtccgccggccgctcatcaacacgatgaacctggccgac
ctgctgccgaccagcaccatctggaccggcgaacgcgccatgccccgatgtacccgccgctgtgccggcgctcatgcgtcacgcaaggatcaacggccgttccggctgaacctgcacgtgccgcga
cctcgggccacaccttttatgttcgggccgaccgggcgcaggtaaatcgacgcacctggcggatcctcgctcgctatgccggcatgtcgatcttcgccttgacaagggcatgtcgatgtaccccgctgg
ccgccggcatccgtgcgggccacgaagggccaccagcgggcctgcacttcaccggtcgccgcgaccgtgcttcgaggccgaacgcctggcgttctgcccgttgcagttcctgagcaccaagggcgaccgtgcttgggcgatgggagtg
gatcgacaccatcctggcgttgaacggcgtcgaaacgaccccgccagcgcaacgaaatcggcaacgcgatcatgagcatgcacgccagcggcgcgcacgctctccgagttcagcgtgacgattcaggat
gaggcgatccgcgaggcgatccgccagtacaccgtcgatggcgcaatgggccatctgctcgacgccgaagaggacggcttggcgctgtccgacttacagtgttcgagatcgaagagctgatgaacctcggcga
gaaatttcgccctgccgtgttgctctacctgttccgccgtatcgagcgcgcccctgacgggccagccggccgtcatcatcctggacgaagcctggttgatgctcggccacccggcattccgcgcgcgaagatcagggaaat

ggctcaaggtgctgcgtaaggccaactgccttgtgctgatggcaacgcagagcctgtccgacgccgccaacagcggcatcctggacgtgatcgtggaatcgaccgcgaccaagatttcctgccgaatatttacgcc
agggatgaggacacggcgggccctgtaccgcccgcatgggcctgaacgctcgccagatcgagattctggcccaggccgttcccaagcgtcagtactactacgtgtcgaaaacggccgccgtctctacgacctggca
cttggcccgctcgcgctcgcgttcgtcgtcggcgcatccgacaaggaatccgtcgccatcatcaagaacctggaagccaagttcggcgaccagtgggtggatgaatgctgcgtggccgggggcctcgccttgatgaat
acctggaggcagcatgagttttgcagacacgatcaagggcttgatcttcaagaagaagcccgcaacggccgcagcagcggcgacgccggcccgcaaaccgacacaaccgtacctgacggcgcgcg
gcgcacctggaacgaccacgttggttccgttgtgtcgcaaaagcagacctggcaggttgtcggcgatcctttcgctgatgatcgtcctcgcggcggtcggcggcatcatccacatcggcagccagtcgaagttcgtgc
cctatgtctacgaggtagacaagctcgggcagacggccgccgtgggccgatgaccaggggcgtcgaaagccgatccgcgtgtcattcacgcctcggtggctgagttcgtcggtgatgctcgcctggtgacgccg
gacgtagctttgcagcgcaaggccgtctaccgcctctatgccaagctcgggccgaatgaccgggccaccgccaagatgaacgaatgccaacgggcaccgcccgttcgctcgcgcggccgtcg
aaacggtcagcaccgaaatcacttccgtaatcccgcagacgcccgacacctggcgattgggtcgagacgcgcgacaggcgaaggcgtggtgaaaggcccagccgtgcgcatgcgggccttggtgac
ggtctacgtcgtcgagccgacgggcggacaccaaggaagaacaactgcgaaacaacccggccgcgggatctacgtccgggacttctcctggtcgaggcactgaattatgaaaaaggaactgtttgcttt
ggtcctggccgcgtccgttagcgtgcctgcatttgccgccgatcccgcgcggaccgactgactgacctctatttttccggcaagaacccggagctgaccgcgcaagagcgggcggcccatcgccatcgccaagaagtg
ggaggcgggtaccgccggcatgcggccggtggttccggtggttccgttcctgttcggcgcgcagcagccgagcatcgtatgcgccgtgctgcaagtgtgcgacgtggccctgcaacccggcga
gcaagtcaacttcgatcaacctgggcgacaccgcccgttggacggtcgaggccggccattaccggcagcgggcgcgaacgaaacccagcgacctcatcatcaagccgatgatgtgggcctggaaaccagcctggtc
gtgaccacggaccgccgcgctaccacatgcgcctgcgctcgcatcgcacgcagtacatgccgcaggtgtcgttcacctacccggaagatgcccttgcgaagtgggacgccatcaagaaccgcgaacagcgggg
atcgcgtcgagaaaaccattccgcagaccggcgagtacctgggcaacctgagcttcaactactcccgtcagcgggtccacgtcgtggaagccggtgcgcgtctacaacgacggcaagaaaaccatcatccagatgc
cgcactcgatggaacagacgaagccgacgctcctggtcgttcgcaggaggaggccggcctgttctccgacgatgaaacggtgatggttcaactaccgggtccaggcgaccgctacatcgtcgatacgattttcg
acaaggccatcctcatcgcgggcgtgggcagccaggacccgcgtgaccatttcaaggggggaactaaaccatgcgtaagattctgaccgtcatcgcactcgcggccgcgttggccggctgcgcgacctccaagt
acggcagcttcgtccaggacgccgccggcgcctacaaccagaccattgcgaccgacgcggtgaagcagctcgtcaagctctaccccgccggcgcaaaccaagctggaattgcagcaggctacgcccgatccgttc
ggcattgccctggtcactgacctttcgcgcccaggggctatgctgtcgagtacaaggcccgacgcggcccgcagctccggctgctgcgtcctcggccgctgcgaagccgcaacgccgcaaggcccagg
gcggctatccgctgcgctacgtgctggaccaattcagcgacacgcaacctgtatcgcctgaccgtcatggtcgctctcaatcgctcacgcgcgcctacctcgcccaaaacaacacgatggtcccgggccgggcgcatg
ggttcggaaggagtaagccaatgagcgaagatcaaatggcgacccgacgcatcgccagatgcggtcaagccgaaaagcgggggttccgccgcgtcaacaacatgccgatgtacctcatcggcggtgtgctcggcatc
ttcctgctggtgatggccctggttgctgcgctgcgcatcgcgctgcgcagaagaaccagccgggagctgcgaaggctgagaaggccgggcagcaccagcatgtttgccgacgaaattgccggcaaacagcaggacggca
tcatcaaggccaagccgctggagatttcgccggaacaaaccgcccagcaaccgacgacggagctgacgccagccccgggcgcagggaacgactatcacggtcgcacgggcccgaggaacctggaccagccccc

gacgccgccgcagggtgcgcgcaacgaggacctggaccgcatccgcatggcgaagttgcagatgctggaagaggcgatcaaggccaagacgacggtgcgcatcgacgccgccgcggcagccaggccagcgc
cggcgcgggtgctccgacgggccgcgaggaaaccctgccgcatccaggagctgcgtcggcaggctgaagaacgcccgccgccaccgatccgaccgccgcctatcaggccgcttgccgcaggctcgcacga
tgggcggcgcgcggcaggggtggcggtatgggcggctcgggtgccgcaccctcgtgcagacctcgaaccgcagtggtggcggcgctggctatgggtcgttcgacaaccgcagcgaggcggcgaccgttggcgg
ctcgactcccagccggaagcacctgcaacgcccttatgtgctgcgcgctggcttcgtcgttccggctacgcttatctcgggcatcaactccgatctgccaggccaaatcatggcccaggtatcgcagtcggtgtacgac
acggcgaccggcaagcacatgctcatcccccaaggctcgcgcctggtgggcagctactcgaacgatgtggcctacgggcagaagcgcgttctggttggcatggcagcgcatcatcttcccgacggcaaggcaat
ggacattgggccatgccgggcggcgatagcgctggttatgcaggcttcaacgacaaggtcaacaaccactacttccgcaccttcgcatccggccgtcgttgcgggcgtcgttgcgggcatcagcttgagtcag
gaccgtggcaacagcaacagcggttacggacagacaagacgcgggttccgcgatgagtgaagcgttgggtcaacagctcggccaagtaacgggcgcagatgatcgccgaaaaacttgaatatcgcgccgacgctgg
aaatccgtccgggctatcgcttcaacgtcattgtcacgaaagacatgacgtttttctaagccctaccaggcgtttgactattaactccaaggagtaacttatgaagaagctcgctaaagtatgctc
tggtgctggccctctcggtcggtcacgccttggcggtcaagcgggcattccggtcatcgacggcaccaacctgtcacaaaccactgtcacccgcgattcagcaggttgcgcagtccagaagcaaatcga
ggaatacccggacgcagttgcagcagtacgaaaacatgctgcaaaacacggtggccccggccgctacgtgtgggaccaggcgcagtccaccattcaacgggcctgatgagcgccgttgatacctgaactactaca
agaaccaggcgggcagcatcgacgcttacctgggcaagttcaaggagggtcgccgtgcttctcctgtcgggctgctcggaaagcgagcgcaagggcgatggaagagaaccgccgcctg
gcgtccgaatcgcagaaaaaggccaacgatgcgctgttccgtggcctcgatcagcagcagagcaacctcaagtccgacgccgccacgctggagcaattgaaggccaaggccgcacggcgcaggccagttg
gaagccctcggctacgccaaccagttcgccagccaggccaaccagctcatgcaaatccgttggcctctgcttgcgcagcagaacgccatcgccacgcagatgcaggcccaggccgacgaccgcccagc
aggacgctgcgggcgcgaagctgcgcgaggttctgtaccgccgcaagcccgtctaagacctggttgccgctggccgccgtcatggcggccag
cctggcaggctgcgacaacaagcccgacaccgacaagctgacctgccgcgatctgccgaaggtcacggatgccgctcaacgcgcggaggctgttgaagaagtgcccgcggcggagaaccgggaggcttcaagcc
cagcgaaaagaaaagagtggtgatgacgtatgaaaatccagactagagctgccgcgctcgcggtcctgatgctggccttgatgccggtagcggcatacgcccaaatccgacaatttcgggcatcctcgacaacgtattg
cagcgctaccagaacgccgcgagcggctgggccactgtcgtccagaacgccgcaacctggctgttctggaccttgaccgtgattagcatggtctggaccttccggcatgatggcactgcgcaagggccgacatttggc
gagttcttgccgagttcgtgcggttcaccatcttcaccggcttcttctggtggctgctgaccaacggcccgaatttcgcgtcgtccctgcggcagattgcaggccaggcaacgggggttgggggcagg
ggctttcgccgtccggcatcgtcgatgttggcttcgagatttcttcaaggtgatggacgaaacctcgtactggtcgccggtcgataggcttcgtcggtgcctcgttgggcgggccgccatcctctgcatcctggccctggtcg
gcgtgaatatgcttctgctccctggcgtccggatggattcttgcctacggcggtgtgttcttcctgggcttcggcggctcgcgctggacctcggacatggcgatcaacactacaagaccgtcctcgggggtcgccgcgca
gctcttcgcaatggtgctgctcgtaggcatcggcaagaccttcctcgatgactactacagccgcatgagcgaaggcatcaacttcaaggaacttggagtgatgctgatcgtcggcctgatcctgctcgttctggtcaaca
aggtgccgcagctcatcgccggcatcatcaccggcgcgagcgtcggcggtgctggtatcggccagttcggcgctgtggcggccgcgacggcccggcgcggcaatcgcaactggcggcgcgcat

	ctatcgcggccggcgctgcggcggccggtggcgcaggccatcatgcggccgcgtcgaaggccagcgataacgtctctgccggcactgacattctgtcgagcatgatggcggcggcggtggcggcg
	gcggtggtagccgccgcaccagcggcggcgacgggcgtcgggtggccgggtgtctcgggcggcggtgaaacccgatggcctcggccgccgcacaacagcagcgcgcacagcgggcagt
	tcgggcggcggctcggtggtggtgccgttcgtctgccacgcggccaagggcggccggatcgcggccgatacctgccaacctggcgaaaggtgccggctcgattgccaaggccaaggccc
	ggcgaaatgcgcgcatcggcccaggaacgcatcggcgataccgtaggcggcaagatcgccgcgatcaggcaatttcgcggcgcgggtgcggcggccgcagaccgctgcaaccgtcgccgatagcaacagccaggcgc
	aggaacaacctgcaccggcacccgcaccgtcgttcgacgacaacaacagggaagcggccgccgacgcggattccgaaagtggcgagcttcgtcaacaaagcccgcccaatcctga
14	tra1 module traFG-traJXIH-traKLM RK2
	tcaccaggtcagaaccggcctgatgacggtgatgatttgcgaacgattgacaggcccgaagtagcggccgtcgaaagacgtgtcgcttacgtcggacataagcagaacctcggcggtccccaggggtgtagctgtc
	ggactgataacgaggcagcggccgtcctgatggatcgggccttgatgaggcagcgccgccattcacgccgcacgccggcgtcggtgatggcaacctcgtcgccttagcggctaaaactcgctt
	catcatgtagccgtagtcgccgggggcagaaaccgccggcgatgtagccccgctccttggcgtccgaaaacacgccgacttgcggcgcgggcagaacatgacgtaagccccccttctccaccgggcgcattcgatttcca
	gtacaggccgaccggaatgcttttggtggtgttgacctccgcgccggcgagataggccgcgccggcgagcaacaaggccgccgcctccgatggcgacgtactggtgaggcgctggaagcggctcatatcgt
	gatcccctcccctcctcgacggtggccgtctggacctccggagccggtacggccgcgcgggcctggaatatcgggtctttgaagtagagcggctgcttgccgtagatcgcgggatagccgg
	cgacgtacacaaccatgtcgcccgcctcttcaatgctgccgtcggccccggcgcatgcgcaggcattcatcgggggtcagcaatggccgctgcactcctgagaggtccgcgagacgttgcccaac
	agcgccgacgtgcggcgccgctcgtcgtgatctgctcctcacgatggtcgtcgtgtcagtttttgacaggtgctcggccgtctcctccacgcgggttcgcacgttcgacgtgatg
	ctttcgtcgtggccgtagccggtttccgcggctcttgagctggttaatgtcctggcagatgaggtagcacttgatgccgtagccggcgacgaaggcaatgggactcttgcaggatttcgagcttgccccaggctgggggaac
	tcgtcgagcatcatcagcagacgatgcttgtagtgcgcgacaggacggccgttctcgaagtccatcttgtcggccagcagccgcgacgatcatgttgaccatgacgcgcacccagaggccttgtcgt
	tgggctgcgtcacgatgaacaggcttaccgggtcgtcgtggtgcatcagttgcttgatgcggaagtcggacttgctgacgttgcgggccacaaccgggtcgcggtacagggccaggtaggacttggcggtggaca
	gcacggaaccggatctttcctccgggcggtccatcatgtcgcgggccgcgagagccgacccgacgaggtggttctgcccgtcaacgtggccgtaggtggtcatttccatccaaagctcgcccacgtcgcggttcgggtc
	ggcaagcatgccgtccaccgacgggcaggtggccggcgtaccctcgttcttagccttgtagagcgcgtgcaggatgacgccgcctggctggttttctgccagtgcgattccaggccctgccgtcc
	ggatcgacgatcaggggtggcaaggttctgcacgtcgccaacctcggtactggtcccaagcggatttcatcgagcgggttccagcacgcgctaccctgcgcggatgccggctcaaagcgcacgacctgttgcgg
	gcatgcttcttccgccagccggcggtcagcgccccacaactcgccttttcaggtcggtgatgacggcgctgtgcgcccaggaaagcagcgtcggaacgaccaggccgacgcccttgccgaggcgcgtcggcggcgt
	aggtcaagacgtgctcgggggccgttgtgccgcaggtagtggaacttgccgtcctgtcctgccagccgcccacatagacgccgctggaagtgggcgggtgtttgccctgacacgaccagctcgacgacggtgcgcggccc

ggggcagcaggccggcggcctgtatgtccttcttgtcggcccagcgggccgaaccgtgcagatagtcgttcgcctgccggtgttcgccttgaccatctgcgtgacggccgtgcccagcaggcccacggtcgaaa
cgaccatacccatgctggccgcgcgcgcatgaaatcgtcgggatattggccgtaccacttgccgggcccattgaaggatcgaccagggcgtgtagacgtggttgatattccagccaagtccggcctgatactggaagga
atgggcgaaatattgcgtcgcggtctgcaagcctgcccccaagggacagcggccggcgaggatgtggaacggtcttgctgtggccttcgggttttttcgcccgtatctgtggcccccacggcgttgtttcggttcttccatctactcct
acctcgggtagttttaagggagcctcgcgggggtcacggtgacgaggatcaccgatggcgaggcgcttcatgcgttgcaccgtggccttatcgacgggcagcaccagaatctcgtcgtttcttttcctcaacagggcca
gcgcctggtcctcgacgttccgggtgcctgcatagaggacagcgcgcaccaacataatcagtatatatcgtgcatgcttcggtatatcgaagccgtttagccgctttgctcgcgctcggcaacatatttctcggccgccgcgatc
tgttcgggcttagccctcttcctggcccagaaactcccgtcgcagtgcgtgagctggttcggctccttgctgctcccacgtgaccaggaacatcacgcggcaatagcatttcagctccgccgcggcgatgcgaaccaca
ccgagttgggacagcgctcgcaaacggttttggcttcgtccaatgcgtccaacgttgggcttgcgggagtgcgacggttccgccggcgctgacggcgcgagcgtcccgttcgatcgcgtcg
ccgcctgtgggcgttgagggtggttctggctgcgggcaggtcgaatgcctccatcgccgccgcgatctcttcgtccgtcatttcgttccgctcgatgtgcttgctcctcgtcagtgattgttcttgacggcgaggctcaagg
gcggcgtcgtcaaaggtgattgccagacggccagcggcggccgcctgcgcgatccgctcctgaactctgctgtgccgttgacggtgatccggtgccgaagcgctccattgccaggcgcagggcggcgtccag
gccgtccgtggtggcctcgcgcgagaacttgcaggcggtcgctgtcgcggacgcgatagatgatggttccttcttcgtgatgttgtccgtcacggccgcatggcccggcttggcctcg
ccgctgccctggatggtgttgccctgcggccctcgcgtgcgcgcagcgcggccaggggccttgtcgtcgcccttcatcgcctcggccttgagccagtcgcggcccacgcgcggctgcgtcgctcct
ggaccgcctgacggccctgccggtactcgcggttgatcttgtccaggtcgaggccttgtgcggcctgcgcgtacatcagtcgcttgcaatgcgcccctcgcccagcagcttgataggcggcgcggcgca
gccggttgctgcgcatcgcggcttcaatcaggcggtcacgacgccgcgcagcgtgtccagctcgccctgcgccccccatttcctggcgttcagactgatacccgggcgtatagctcggtggtgtcgatggcg
ggtcttgagcggcgcttgctcgatactcccgccgccgggggggcttcgccgccctcgggctgcgaatcgggcttcgagcttcggcctggacaggtgctggccttgaccgtcgtg
ccgtcgccggcctcgaagatgaagccgtttccgcgctcgcgcagcttaagcccgtttttcccgcaggacgcggtgcaggtcctcccaggattgcgccgcttgcagctccggcaggccgattccagccga
ccaggctttccacgcccgcgtgccgctccatgtcgttcgcggcggttctcggaaacgcgctgccgcgtttcgtgattgtcacgctcaagcccgtagtcccgttcgagcgtcgcgcagcgaggcgcggta
ggcccgatacggctcatgtggtgtttcgggtgaatcttgttgatggcgatatgtgcaggttgtcgtgtcgtgatgcacggcactgacgcgctgatgctcggcgaagccaagcccagcgcagatgc
ggtcctcaatcgcgcgcaacgtctccgcgtcgggcttctctcccgcgcggaagctaaccagcaggtgatagtcttgtcggcctcgggaacgggtgttgccgtgctggccatcacctcggccatgacagcgggg
cagggtgtttgcctcgcagttcgtgacgcgcacgtgacccaggcgctcggtcttgcctgctcgtcggtgatgtacttcaccagctccgcgaagtcgctcttcttgatggagcgcatgggggacgtgcttggcaatcacg
cgcacccccccggccgttttagcggctaaaaaagtcatggctctgccctcgggcggaccacgcccatcatgacctgccaagctcgtcctgctcttctttcgatcttcgccagcagcgggcgaggatcgtggcatcaccgaa
ccgcgccgtgcgcgggtcgtcggtgagccagagtttcagcaggccgcccagggcggcccaggtcgccattgatgcgggccagctcgcggacgtgctcatagtccacgacgcccgtgatttttgtagccctggccga
cggccagcaggtaggccgacagggctcatgccggccgccgccgccttttcctcaatcgctcttcgttcgtctggaaggcagtacaccttgataggtgggctgcccttcctggttggcttgatttcatcagccatccgcttg

	ccctcatctgttacgccggcggtagccggccagcctcgcagagcaggattcccgttgagcaccgccaggtgcgaataagggacagtgaagaaggaacacccgctcgcgggtgggcctacttcacctatcctgccc
	ggctgacgccgttggatacaccaaggaaagtctacacgaaccctttggcaaaatcctgtatatcgtgcgaaaaaggatggatataccgaaaaaatcgctataatgaccccgaagcagggttatgcagcggaaaagc
	gctgcttccctgctgttttgtggaatatctaccgactggaaacaggcaaatgcaggaaattactgaactgaggggacaggcgagagacgatgccaaagagctacaccgacgagctggccgagtgggttgaatcccg
	cgcggccaagaagcgccggcgtgatgaggctgcggttgcgttcctggcggtgaggcggcgttagcgtccggctatgcgctcgtcaccatttgggagcacatgcgggaaacgggaagcgtc
	aagttctcctacgagacgttccgctcgcacgccaggcggcacatcaaggccaagccgccgatgtgcccgcaccgcaggccaaggctgcggaacccgccgccggcacccaagacgccggagccacggcgccc
	gaagcaggggggcaaggctgaaaaagccggccccccgctgcggcccccgaccggcttcaccttcaacccaacaccggacacaaaaaggatctactgtaatggcgaaaaattcacatggttttgcagggcaaggcgggg
	tcggcaagtcggccatcgccgcgatcattgcgcagtacaagatggacaagggggcagacacccttgtgcatcgacaccgcacccggtgaacgccgacgttccgaacgtccgccggctgaa
	catcatggccgccggcgacgaaattaactcgcgcaacttcgacacccctggtcgagctgattgcgccgaccaaggatgacgtggtgatcgacaacggtgccagctcgttcgtgcctctgtcgcattacctcatcagcaacca
	ggtgccggctctgctgcaagaaatgggcatgagctggtcatccatacccgtcgtcaccggcggccaggctctcctggacacggtgagcggcttcgccccagctcgccagcccagttcccggccgaagcgctttctgtg
	gtctggctgaacccgtattgggggcctatcgagcatgaggcgcaagagagcttgagcagatgaaggcgtacacggccaacaaggcccgcgtgtcgtcgtccatcatccagattccggcccctcaaggaagaaaacctacggc
	cgcgatttcagcgacatgctgcaagagcggctgacgttcgaccaggcgctggccgatgaatcgctcacgatcatgacgcggcaacgcctcaagatcgtgcggcgctgtttgaacagctcgacgcggcggc
	cgtgctatgagcgaccagattgaagagctgatccgggagattgcggccaagcacggcatcgccgtcggccgcgacgaccggtgctgatcctgcataccatcaacgcccggctcatggccgacagtgcggccaa
	gcaagaggaaatccttgccgcgttcaaggaagagctggaaggggatcgccccatcgttgggggcgaggacgccaaggccaaagcggaggcgatgctgaacgcggcccctgggcggccagcgacaaggacgcaatggcg
	aaggtaatgaaggacagcgccgcgcaggccgatccgcaggggaaatcgacgacggccttggccgccagctcgccggccaaggtcgccggacgcgcggcgcgtggcgatgatgaacatgatcgccg
	gcggcatggtgttgttcgcggccgccctggtggtgtgggcctcgttatgaatcgcagaggcgcagatgaaaaagcccggcgttgccgggcttgttttt
15	*Sal-crRNA Array 1 (522bp)*
	TTTGAAAACAAAGAATTAGCTGATCTTTAATAATAAGGAAA*TGTTACATT*AAGGTTGGGTTGTTTTTATGGGAAAAAATGCTTTAAGAACAAATGTATACTT
	TTAGAGAGTTCCCCGCGCCAGCGGGGATAAACCG**GCTTTAAGCGTTAGCTCCCCCA**TTCTGCTCCCCGAGTTCCCCGCGCCAGCGGGGATAAACCG**GTACGTTAG**
	CGTATATTGATGCCGCAGAGACGGAGTTCCCCGCGCCAGCGGGGATAAACCG**GCGATAACTGGACAGTTTTATCCGCCGAGCAT**GAGTTCCCCGCGCCAGCG

	GGGATAAACCGGGCGCACTGGATGCGATGATGGATATCACTTGGAGTTCCCCGCCTCTGCGGTAGAACTCCCAGCTCCCATTTTCAAACCCATCAAGACGCCTT
	CGCCAACTCCTTCACCAGAGGTAGCATTATCCGCATAACGTCACGGCAGCGACGTTCTATTCTTCCAGGAAGAGCCTTATCAATATGTTGGTGATTATCCAGTCTT
16	Repeat Sequence
	GAGTTCCCCGCGCCAGCGGGGATAAACCG
17	Spacer Sequence for targeting the *Salmonella invB* gene
	GCTTTAAGCGTTAGCTCCCCATTCTGCTCCCC
18	Spacer Sequence for targeting the *Salmonella sicP* gene
	GTACGTTAGCGTATATTGATGCCGCAGAGACG
19	Spacer Sequence for targeting the *Salmonella seeE* gene
	GCGATAACTGGACAGTTTTATCCGCCGAGCAT
20	*invB* from *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2; spacer sequence in bold
	atgcaacatttggatatcgctgaattagttcgttccgcactggaagtaagtggttgcgatccttcattaattggaggaatagatagccattcaacaattgttctggatttatttgcattgccaagtatctgtat
	cagcgtcaaggacgatgatgtatggatctgggcgcaattgggtgctgacagcatggtggttattacaacagcgggcttatgaaatcttaatgaccataatggaaggatgccatttgcccgcggcgggg
	caattactactg**ggggagcagaatgggggagctaacgcttaaagc***ctta*gtgcatccggatttttatctgacggtgaaaagttctctactgccttgaatgggttttacaactatctggaagttttagtc
	ggtcgctaatgagatga
21	*sicP* from *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2; spacer sequence in bold
	ttgcaagcacaccaggatattatcgctaatattggtgagaaattgggtttaccgctcactttgacgacaacaatcagtgcttattattactcgatagcgatatttttacgtctattgaagctaaagatgatat
	ctggttattgaacggtatgattataccgttatcgcctgtttgtggcggcagattatggtgattaatggtgaactggctgcgaataaatg*aag***gtacgttagcgtatatttgatgccgca**
	gagacgttgttgcttatacatgcaattaccgatctgacaaatacttaccatattatatcgcagcttgagtcatttgtgaatcagcaggaagcgctcaaaaacatactgcaggaatatgctaaagtatga
22	*seeE* from *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2; spacer sequence in bold

	atggtgcaagaaatagagcaatggttacgtcggcatcaggtgtttactgagcctgcatatttaggggagaccgccatattacttgggcagcagtttatattatcgccttacctggtgatctatcgtattga ggcaaaagaaatgattatttgtgagttcaggcgcctgacgcccgggcaacctcgaccacagcaattgttcacttactgggactttacgcgggatatttgtgcatcacccgcagttaacatgtttaaag atgttgataatcaccgacgttctggatgaaaaaaaagccatgctacgcaggaaattattgcgcatcctgacagtaatgggagcgacctttacacagcttg*atg***gcgataactggacagtttatccg** **ccgagcat**cttatccagcgacgtttttaa

Table 7: Function and origin of each genetic element of the pFS-Sal-09-proAB-rm plasmid containing a Type I CRISPR/Cas system.

Component	Location	Size (bp)	Origin	Function
J23114 promoter	1-29	29	Synthetic promoter	Initiates transcription of Cas3 and Cas3 cascade
Cas3 cascade (*casABCDE*)	105-4455	4351	*E. coli* K12	Recruits Cas3 and recognition of PAM
Cas3	4472-7138	2667	*E. coli* K12	Nuclease activity; cleavage of DNA
Promoter-crRNA array	7182-7210	29	*E. coli* K12	Initiates transcription of pre-crRNA array to mature crRNA array
invB-cr4	7278-7309	32	Synthetic sequence	Spacer sequence of guide RNA. Binds to the complementary target DNA sequence of *invB* gene in *Salmonella enterica*
sicP-cr1	7339-7370	32	Synthetic sequence	Spacer sequence of guide RNA. Binds to the complementary target DNA sequence of *sicP* gene in *Salmonella enterica*
sseE-cr2	7400-7431	32	Synthetic sequence	Spacer sequence of guide RNA. Binds to the complementary target DNA sequence of *sseE* gene in *Salmonella enterica*
oriT	7661-7781	121	RP4 plasmid	Origin of transfer. Transfer of CRISPR/CAS3 system to recipient
rrnB T1 terminator	7809-7895	87	*E. coli rrnB* gene	Transcription termination
p15A ori	8064-8609	546	pZA31MCS vector	Plasmid replication
Lambda T0 terminator	8723-8817	95	Phage lambda	Transcription termination
proA	8837-10090	1254	*E. coli* K12	Proline biosynthesis
proB	10102-11205	1104	*E. coli* K12	Proline biosynthesis
proAB promoter	11250-11278	29	*E. coli* K12	Initiates transcription of *proAB*

Table 8. Conjugation results in JM109. Conjugation from S17-1::pFS-EcoCas3-01-rm, S17-1::pFS-Sal-09-rm, S17-1::pFS-EcoCas3-03-rm and S17-1::pFS-Sal-08-rm, in *E. coli* JM109 strain showed a comparable efficiency between the two constructs and the respective control plasmids. Conjugation efficiency is calculated dividing the number (CFU/mL) of transconjugants by the number of recipients.

Donor	**Recipient**	**CFU/mL**	**Conjugation Efficiency**
S17-1::pFS-EcoCas3-01-rm	JM109	1.2 x 107	1.4 x 10-2
S17-1::pFS-Sal-09-rm	JM109	7.0 x106	7.4 x 10-3
S17-1::pFS-EcoCas3-03-rm	JM109	8.1 x 106	3.4 x 10-2
S17-1::pFS-Sal-08-rm	JM109	1.5 x107	2.9 x 10-2

Table 9. Conjugation results in FS26. Conjugation from S17-1::pFS-EcoCas3-01-rm, S17-1::pFS-Sal-09-rm, S17-1::pFS-EcoCas3-03-rm and S17-1::pFS-Sal-08-rm in *S.* Enteritidis strain FS26 were carried out in two biological replicates. Results showed significant reduction in *S.* Enteritidis strain FS26 by conjugation with the active constructs, when compared to the respective control plasmids. Conjugation efficiency is calculated dividing the number (CFU/mL) of transconjugants by the number of recipients.

Replicate	**Donor**	**Recipient**	**Transconjugants (CFU/mL)**	**Conjugation Efficiency**
1st	S17-1::pFS-EcoCas3-01-rm	FS26	7.6 x 106	1.2 x 10-2
	S17-1::pFS-Sal-09-rm	FS26	7.0 x102	2.7 x 10-6
2nd	S17-1::pFS-EcoCas3-01-rm	FS26	1.3 x 107	1.0 x 10-2
	S17-1::pFS-Sal-09-rm	FS26	1.0 x103	1.2 x 10-6

1st	S17-1::pFS-EcoCas3-03-rm	FS26	3.0 x 106	1.0 x 10-2
	S17-1::pFS-Sal-08-rm	FS26	3.7 x102	2.7 x 10-6
2nd	S17-1::pFS-EcoCas3-03-rm	FS26	1.0 x 107	2.9 x 10-2
	S17-1::pFS-Sal-08-rm	FS26	2.0 x103	5.6 x 10-6

Table 10. Selected spacer conservation in *Salmonella*

Spacer	PAM	# hits in *S. enterica*	conservation level in *Salmonella*				non-*Salmonella* hits
			100	≥95	≥90	<90	
invB	AAG	10613	96	2	2		23
sicP	AAG	10610	74.5	20.7	4.7	<1	46
sseE	ATG	10604	68.1	30	1.9		52

Table 11

Age (days)	Under lamp (°C)	Ambient temperature in room (°C)	Relative humidity (%)
Up to 1	35	25-30	60-80
1-7	32	22-27	60-80
7-14	29	19-25	40-80
14-21	26	18-25	40-80
21-28	24	18-25	40-80
28-35	-	18-25	40-80
Over 35	-	15-25	40-80

Table 12. Plating protocol

Plating for Salmonella	**Enrichment for Salmonella**
XLD + Nalidixic acid (25µg/mL)	Selenite broth, Plate onto XLD +Nalidixic acid (25µg/mL)
Direct counts - Salmonella	Enriched counts - Salmonella

CLAIMS:

1. A non-medical method for enhancing the growth or weight of an animal (optionally a livestock animal), wherein the method comprises the administration of a plurality of carrier cells to the animal, wherein the animal comprises bacterial target cells and each carrier cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the agent, wherein first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the animal or reducing the growth or proliferation of target cells and enhancing growth or weight of the animal.

2. The method of claim 1, wherein the method improves feed conversion ratio (FCR) in the animal.

3. The method of Claim 1 or 2, wherein the target cells are *Salmonella* cells.

4. The method of Claim 3, wherein the target cells comprise *S enterica* and/or *S typhimurium* cells; optionally wherein the *S enterica* is *S enterica* subspecies *enterica*.

5. The method of any preceding Claim, wherein the method kills a plurality of different *S enterica* subspecies *enterica* serovars; optionally wherein each serovar is selected from the group consisting of Typhimurium, Enteritidis, Virchow, Montevideo, Heidelberg, Hadar, Binza, Bredeney, Infantis, Kentucky, Seftenberg, Mbandaka, Anatum, Agona and Dublin.

6. The method of any preceding Claim, wherein the carrrier cells are *Enterobacteriaceae* cells, optionally *E coli* cells (such as F18, Nissle or S17 *E coli* cells).

7. The method of any preceding Claim, wherein the method reduces target cells in the gastrointestinal tract of the animal; optionally wherein the animal is a bird and the method reduces target cells in the caecum, crop, liver, spleen and/or ileum of the bird.

8. The method of any preceding Claim, wherein the first DNA is comprised by a plasmid, wherein the plasmid comprises a RP4 origin of transfer (oriT) and/or a p15A ori.

9. The method of any preceding Claim, wherein the agent comprises one or more components of a CRISPR/Cas system that is operable in a target cell to cut a protospacer sequence comprised by

the target cell, optionally wherein the target cells comprise first and second strains of a bacterial species and each strain comprises the protospacer sequence, wherein cells of the strains are killed.

10. The method of Claim 9, wherein the system is operable to cut at least 3 different protospacer sequences comprised by the target cell genome.

11. The method of Claim 9 or 10, wherein each or some of said protospacer sequences is comprised by a pathogenicity island that is comprised by the target cell.

12. The method of any preceding Claim, wherein the agent
    (a) comprises a guided nuclease that is capable of recognising and modifying a target nucleic acid sequence, wherein the target sequence is comprised by an endogenous chromosome or episome of the target cells but is not comprised by the carrier cells, wherein the nuclease modifies the chromosome or episome to kill the target cells or inhibit the growth or proliferation of the target cells; and/or
    (b) encodes a guide RNA or crRNA of a CRISPR/Cas system that operates with a Cas nuclease in the target cells to cut a protospacer sequence comprised by the target cells.

13. A carrier cell for use in the method of any preceding Claim, wherein the cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a bacterial target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the antibacterial agent, hereby killing the target cell, wherein the target cell is a *Salmonella* cell and the carrier cell is an *Enterobacteriaceae* cell.

14. A composition comprising a plurality of carrier cells for use in a method comprising administration of the cells to a subject to treat an infection by pathogenic bacterial target cells, wherein each carrier cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the agent, wherein first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the subject or reducing the growth or proliferation of target cells, wherein the target cells are *Salmonella* cells and the carrier cells are *Enterobacteriaceae* cells.

15. A non-medical method of killing zoonotic bacterial target cells in an animal (optionally a livestock animal), the method comprising administering to the animal a plurality of carrier cells,

wherein each carrier cell is a bacterial cell comprising a first episomal DNA encoding an antibacterial agent that is toxic to a target cell but is not toxic to the carrier cell, the carrier cell being capable of conjugative transfer of the DNA into a target cell for expression therein of the agent, wherein first DNA is transferred from carrier cells into target cells for expression therein to produce the antibacterial agent, thereby killing target cells in the subject or reducing the growth or proliferation of target cells, wherein the target cells are *Salmonella* cells and optionally the carrier cells are *Enterobacteriaceae* cells.

16. The composition of Claim 14 or the method of claim 15, wherein the method reduces *Salmonella* in the gastrointestinal tract of the subject.

17. The method of claim 14 or 16, wherein the method is carried out on a group (optionally a flock or herd) of animals, wherein some or all of the animals comprise target cells, wherein spread of cells of the target species is reduced in the group; or wherein spread is reduced from the group to a second group of animals.

18. The composition or method of any one of Claims 14 to 17, wherein the target cells comprise different *Salmonella* spp. types that are killed.

19. The cell, composition or method of any one of Claims 13 to 18, wherein the carrier cell, target cell(s) or DNA is respectively a carrier cell, target cell(s) or DNA as defined in any one of Claims 1 to 12.

20. A DNA for use in the method of claim 12, wherein the DNA is capable of being introduced into a target cell, wherein the DNA encodes a plurality of guide RNAs or crRNAs of a CRISPR/Cas system wherein the guide RNAs or crRNAs are operable with Cas nuclease in the target cell to recognise a plurality of protospacer sequences comprised by the target cell genome, wherein the target cell is a *Salmonella* cell and
    (a) the protospacer sequences comprise one or more pathogenic island nucleotide sequences of the target cell genome;
    (b) the protospacer sequences comprise one or more invasion gene sequences of the target cell genome;
    (c) the protospacer sequences comprise one or more secretion system gene sequences of the target cell genome; and/or

(d) the protospacer sequences comprise one or more nucleotide sequences of genes selected from A gene selected from *avrA, sptP, sicP, sipA, sipD, sipC, sipB, sicA, invB, ssaE, sseA, sseB, sscA, sseC, sseD, sseE, sscB, sseF, sseG, mgtC, cigR, pipA, pipB, pipC, sopB* and *pipD* (optionally selected from *invB, sicP, sseE, pipA, pipB, pipC, hilA, marT* and *sopB).*

21. The DNA of claim 20, wherein the DNA is comprised by a plasmid which comprises an origin of transfer (oriT) and an origin of replication (oriV) that is operable for replication of the DNA in a bacterial host cell.

22. The DNA of claim 20 or 21, wherein the DNA comprises SEQ ID NO: 15, optionally wherein the DNA is comprised by a plasmid in a carrier bacterial cell for conjugation to a *Salmonella* target cell.

23. The DNA of claim 20 or 21, wherein the DNA comprises CRISPR repeat and spacer sequences, wherein
    (a) the repeat sequences each comprise SEQ ID NO: 16; and/or
    (b) the spacer sequences comprise one, two or three sequences selected from SEQ ID NOs: 17-19 and complement sequences thereof;

    optionally wherein the DNA is comprised by a plasmid in a carrier bacterial cell for conjugation to a *Salmonella* target cell.

24. The DNA of claim 23, wherein the DNA comprises (optionally in 5' to 3' order) SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19.

25. The method, cell, composition or DNA of any preceding claim, wherein the target cell is an *Enterobacteriaceae* cell (optionally a *Salmonella* cell) and said DNA is comprised by a plasmid, wherein the plasmid is selected from an *IncFI, IncFII, IncFIII, IncFIV, IncFV, IncM, Inc9, InclO, Incl, IncA, IncB, IncC, IncH, IncIa, InclIc, IncI2, IncIy, IncJ, IncL, IncN, Inc2e, IncO, IncP, IncS, IncT* and *IncW* plasmid.