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(54) Title: COMPOSITION, PROBIOTIC FORMULATION AND METHOD TO REDUCE BACTERIAL INFECTION IN
POULTRY

(57) Abstract: The present disclosure relates to compositions and probiotic formulations of bacterial isolates, and methods for se-
lecting bacterial isolates capable of reducing levels of *Salmonella* and/or *Campylobacter* in poultry. Methods for reduction of *Sal-*
monella and/or *Campylobacter* in poultry are also part of the invention.



COMPOSITION, PROBIOTIC FORMULATION AND METHOD TO REDUCE BACTERIAL INFECTION IN POULTRY

Reference to a deposit of biological material

5 This application contains a reference to a deposit of biological material, which deposit is incorporated herein by reference. For complete information see Example 1.

Reference to sequence listing

10 This application contains a Sequence Listing in computer readable form. The computer readable form is incorporated herein by reference.

Index to sequence listing:

SEQ ID NO: 1 is 16S rDNA of NRRL B-50962 (*Pediococcus acidilactici* 64C1).

SEQ ID NO: 2 is 16S rDNA of NRRL B-67320 (*Lactobacillus salivarius* 64C2).

15 SEQ ID NO: 3 is 16S rDNA variable region of NRRL B-67178 (*Lactobacillus salivarius* 39B1).

SEQ ID NO: 4 is 16S rDNA variable region of NRRL B-67179 (*Pediococcus pentosaceus* 39B2).

SEQ ID NO: 5 is 16S rDNA variable region of NRRL B-67177 (*Lactobacillus salivarius* 59B).

20 SEQ ID NO: 6 is 16S rDNA variable region of NRRL B-67180 (*Lactobacillus salivarius* 34B).

FIELD OF THE INVENTION

25 Compositions, probiotic formulations and methods are disclosed, including compositions and probiotic formulations of bacterial isolates, methods for selecting bacterial isolates capable of reducing levels of *Salmonella* and/or *Campylobacter*. Finally, methods for reduction of bacterial infections in poultry are disclosed.

BACKGROUND OF THE INVENTION

30 *Salmonella* and *Campylobacter* are bacterial food borne pathogens common in poultry. These bacteria account for tens of thousands of cases of food borne illness in the United States annually. The USDA Food Safety and Inspection Service regulate poultry processing and inspect poultry processing to reduce the incidence of carcass contamination. *Salmonella* and/or *Campylobacter* contamination within a poultry processing plant are often the result of ante mortem infection of the poultry. During poultry processing the crop of the chicken is often ruptured. As the crop is often contaminated with both *Salmonella* and
35 *Campylobacter*, it is the rupture of the crop that is the primary point from which poultry carcasses are contaminated.

Ante mortem intervention strategies exist that attempt to reduce or eliminate *Salmonella* and/or *Campylobacter* from carcasses. Chemical intervention methods are primarily composed of organic acids or volatile fatty acid compositions. Organic acids are capable of reducing these pathogens from the crop of poultry, but they can cause water refusal in the poultry. To reduce *Salmonella* and *Campylobacter* in the crop, the organic acids are added at elevated concentrations, typically in a concentration of approximately 0.5%. This concentration causes the flock to reduce, or in some cases cease, water consumption. Dehydration, and ultimately, a loss of carcass weight occurs. Animal welfare and economic concerns result. During this period a flock can lose in excess of two percent (2%) body weight. The result is a corresponding loss in carcass yield and a monetary loss to the producer.

Another group of products that can reduce *Salmonella* and *Campylobacter* from the lower GI tract of poultry includes long term probiotic regimes. Existing probiotic regimes are given several days or weeks prior to the slaughter date in order to reduce populations of these pathogens in the lower GI tract of poultry. These probiotic treatments also typically require multiple applications to work effectively.

The successful administration of chemical and probiotic treatment by growers and uptake by poultry is variable. The inconsistent administration is related to the several major shortcomings of current probiotics. For example, current probiotics require multiple applications during the life of a flock to reduce the presence of *Salmonella* at processing. Furthermore, required multiple applications of a chemical or probiotic product typically lead to greater cost of application.

Antibiotics have previously been used to reduce or eliminate *Salmonella* from the gastrointestinal tract of poultry prior to harvest. Antibiotics have withdrawal windows and cannot be used for a period ranging from a few days to a few weeks. Regulatory and social concerns have made the use of antibiotics less prevalent in recent years.

EP 1031287 discloses a poultry drinking water additive comprising monosaccharides and live bacteria of the genus *Bacillus* and a method for reducing harmful bacteria in the intestine of a layer. In US 6,627,188 a cocktail containing water and a carbohydrate is disclosed for reducing bacterial enteropathogens in fowl.

Compositions and probiotic formulations for reducing *Salmonella* and/or *Campylobacter* in animals would be advantageous.

SUMMARY OF THE INVENTION

The invention relates to a method for reducing the level of *Salmonella* and/or *Campylobacter* in poultry meat comprising the step of administering at least one bacterial isolate to the poultry less than 24 hours prior to slaughter such as, e.g., at the time of feed

withdrawal or prior to the feed withdrawal period. The at least one bacterial isolate is preferably selected to reduce *Salmonella* and/or *Campylobacter* in the crop of poultry. In one embodiment the at least one bacterial isolate is from the genus *Pediococcus*, the genus *Lactobacillus* or a combination thereof. In a preferred embodiment the at least one bacterial isolate is from the species *Pediococcus pentosaceus*, the species *Pediococcus acidilactici* or the species *Lactobacillus salivarius*, or a combination thereof.

The invention further relates to bacterial isolates capable of reducing the level of *Salmonella* and/or *Campylobacter* in the crop of poultry such as *Pediococcus pentosaceus*, *Pediococcus acidilactici* or *Lactobacillus salivarius*. Compositions comprising the bacterial isolate according to the invention and an agriculturally acceptable excipient are also disclosed.

An additional embodiment of the invention is a method for selecting at least one bacterial isolate for reducing *Salmonella* and/or *Campylobacter* levels in poultry such as in the crop of poultry comprising the steps of:

- i) identifying a bacterial isolate capable of reducing *Salmonella* and/or *Campylobacter* levels *in vitro*;
- ii) testing said bacterial isolate *in vivo*; and
- iii) selecting a bacterial isolate capable of reducing *Salmonella* and/or *Campylobacter* levels *in vivo*.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 illustrates a flow diagram of a selection process.

FIG. 2 illustrates a flow diagram for a crop assay.

FIG. 3 illustrates evaluation of lactic acid bacteria and isolates on *Salmonella* reduction *in vitro* with a crop assay.

FIG. 4 illustrates evaluation of isolates on *Campylobacter* reduction at 42°C at pH 6.2 for 6 hours in an *in vitro* crop assay.

FIG. 5 illustrates evaluation of isolates on *Campylobacter* reduction at 42°C at pH 7.2 for 6 hours in an *in vitro* crop assay.

FIG. 6 illustrates evaluation of lactic acid bacteria and combinations of lactic acid bacteria isolates on poultry pathogen reduction in an *in vitro* overlay assay.

FIG. 7 illustrates evaluation of lactic acid bacteria isolates on *Salmonella enteritidis* recovery from the crop of broiler chickens after an 8 hour feed withdrawal period in percent *Salmonella enteritidis* recovery.

FIG. 8 illustrates evaluation of lactic acid bacteria isolates on *Salmonella enteritidis* recovery from the crop of broiler chickens after an 8 hour feed withdrawal period illustrates colony forming units per gram (cfu/g; log₁₀).

FIG. 9 illustrates evaluation of candidate lactic acid bacteria on reducing *Salmonella enteritidis* in the crop of 4 week broilers after a 12 hour feed withdrawal period illustrates colony forming units per gram (cfu/g; log₁₀).

FIG. 10 illustrates evaluation of lactic acid bacteria isolates on *Salmonella enteritidis* recovery from the crop of broiler chickens after an 8 hour feed withdrawal period in percent *Salmonella enteritidis* recovery.

FIG. 11 illustrates evaluation of lactic acid bacteria isolates on *Campylobacter jejuni* recovery from the crop of broiler chickens after an 12 hour feed withdrawal period illustrates colony forming units per gram (cfu/g; log₁₀).

FIG. 12 illustrates evaluation of lactic acid bacteria isolates on *Salmonella enteritidis* recovery from the crop of broiler chickens after an 12 hour feed withdrawal period illustrates colony forming units per gram (cfu/g; log₁₀).

FIG. 13 illustrates evaluation of lactic acid bacteria isolates on *Campylobacter jejuni* recovery from the crop of broiler chickens after an 12 hour feed withdrawal period illustrates colony forming units per gram (cfu/g; log₁₀).

FIG. 14 illustrates percent *Salmonella* recovery from the crop of broiler chickens from *in vivo* field trial.

DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the subject matter of the present disclosure, preferred methods and materials are described. For the purposes of the present disclosure, the following terms are defined below.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

The term “about” means a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

Throughout this disclosure, unless the context requires otherwise, the words “comprise,” “comprises,” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

The term “consisting of” means including, and limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present. The term “consisting essentially of” means including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase “consisting essentially of” indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they materially affect the activity or action of the listed elements.

The terms “administering,” or “administer” include providing a probiotic formulation of the disclosure to an animal by oral administration. Oral administration may be via drinking water, oral gavage, aerosol spray or animal feed.

A “decreased” or “reduced” amount is typically a “statistically significant” amount, and may include a decrease that is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 100, 200, 250, 500, 1000 or more times less, including all integers and decimal points in between and above 1 (e.g., 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9), the amount produced by an animal in the absence of a probiotic formulation, for example, water without a probiotic formulation (e.g., the “native” or “natural” levels of *Salmonella* and/or *Campylobacter*). A decrease may include a decrease that is at least one log (Log_{10}) or more less than the amount produced by an animal in the absence of a probiotic formulation, for example, water without a probiotic formulation (e.g., the “native” or “natural” levels of *Salmonella* and/or *Campylobacter* of an animal). A decreased or reduced amount may include a decrease that is at least about 5 fold, 10 fold, 15 fold, 20 fold, 25 fold or 30 fold less than the amount produced by an animal in the absence of a probiotic formulation, for example, water without a probiotic formulation (e.g., the “native” or “natural” levels of *Salmonella* and/or *Campylobacter*).

A “decrease” in a response may be “statistically significant” as compared to the response produced by an animal in the absence of a probiotic formulation, for example, water without a probiotic formulation (e.g., the “native” or “natural” levels of *Salmonella* and/or *Campylobacter* of an animal), and may include a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% decrease, including all integers in between.

“Bacterial isolate” as used herein means a bacterial strain which has been isolated from a live microbial source. A bacterial isolate is thus an isolate of a live bacterial microorganism but not, e.g., an enzymatically hydrolyzed protein, beef extract, yeast extract or autolyzed yeast. Examples of bacterial isolates of the invention include but are not limited probiotics, ie. live microorganisms that are providing health benefits when consumed.

The term “isolated” refers to a material that is substantially or essentially free from components that normally accompany it in its native state. For example, an isolated bacterial isolate may refer to a bacterial isolate that has been purified or removed from naturally or non-naturally occurring components that are present in its naturally occurring environment.

5 The term “modulate” includes to “decrease” one or more quantifiable parameters or indications, optionally by a defined and/or statistically significant amount. By “decrease” or “decreasing,” “reduce” or “reducing,” refers generally to the ability of a probiotic formulation to produce a lesser physiological response in an animal relative to the response caused by a control formulation. Relevant physical responses will be apparent to persons skilled in the art, and may include one or more of a decrease in levels of *Salmonella* and/or *Campylobacter* in an animal. A “decreased” or “reduced” amount is typically a “statistically significant” amount, and may include a decrease that is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50 or more times less (e.g., 100, 500, 1000 times), including all integers and decimal points in between and above 1 (e.g., 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9), the amount produced by an animal in the absence of a probiotic formulation (e.g., the “native” or “natural” levels of *Salmonella* and/or *Campylobacter* of an animal). A decrease may include a decrease that is at least one log (Log_{10}) or more less than the amount produced by an animal in the absence of a probiotic formulation, for example, water without a probiotic formulation (e.g., the “native” or “natural” levels of *Salmonella* and/or *Campylobacter* of an animal). A decreased or reduced amount may include a decrease that is at least about 5 fold, 10 fold, 15 fold, 20 fold, 25 fold or 30 fold less than the amount produced by an animal in the absence of a probiotic formulation, for example, water without a probiotic formulation (e.g., the “native” or “natural” levels of *Salmonella* and/or *Campylobacter*).

As used herein, the terms “quantifying,” “quantification” or other related words refer to determining the quantity, mass, or concentration in a unit volume, of a parameter or indication, for example, levels of *Salmonella* and/or *Campylobacter* of an animal.

As used herein, the term “poultry” refers to domesticated birds. Poultry includes wildfowl, waterfowl, and game birds. Examples of poultry include, but are not limited to, chicken, broilers, bantams, turkey, duck, geese, guinea fowl, peafowl, quail, dove, pigeon (squab), and pheasant.

As used herein the term “agriculturally acceptable excipient” is a natural or synthetic substance formulated alongside the active ingredient of a formulation included for the purpose of long-term stabilization, bulking up solid formulations that contain potent active ingredients, or to confer a therapeutic enhancement on the active ingredient in the final dosage form, such as facilitating absorption, reducing viscosity, increasing viscosity, or enhancing solubility. In this context excipients may include but is not limited to skim milk powder, inulin, dextrose, vegetable protein powder, yeast extract, gums, citric acid, sodium

ascorbate, wheat bran, rice hulls, calcium carbonate, chlorine binding agents, sugars, proteins powders, corn starch, starches, oligosaccharides, nitrite, nitrate, buffering agents, acidifying agents, etc.

5 DETAILED DESCRIPTION OF THE INVENTION

The following description is merely exemplary in nature and is not intended to limit the present invention, its applications, or its uses. It should be understood that throughout the drawings, corresponding reference numerals indicate like or corresponding parts and features. The description of specific examples indicated in various embodiments of the present invention are intended for purposes of illustration only and are not intended to limit the scope of the invention disclosed herein. Moreover, recitation of multiple embodiments having stated features is not intended to exclude other embodiments having additional features or other embodiments incorporating different combinations of the stated features.

Furthermore, the detailed description of various embodiments herein makes reference to the accompanying drawing FIGS, which show various embodiments by way of illustration. While the embodiments are described in sufficient detail to enable those skilled in the art to practice the invention, it should be understood that other embodiments may be realized and that logical and mechanical changes may be made without departing from the spirit and scope of the present invention. Thus, the detailed description herein is presented for purposes of illustration only and not of limitation. For example, steps or functions recited in descriptions, any method, system, or process, may be executed in any order and are not limited to the order presented. Moreover, any of the step or functions thereof may be outsourced to or performed by one or more third parties. Furthermore, any reference to a singular component may include plural embodiments, and any reference to more than one component may include a singular embodiment.

Methods for reduction of *Salmonella* and/or *Campylobacter* in poultry

The invention relates to a method for reducing the level of *Salmonella* and/or *Campylobacter* in poultry meat comprising the step of administering at least one bacterial isolate to the poultry less than 24 hours prior to slaughter of said poultry preferably at the time of feed withdrawal or during the feed withdrawal period. Preferably the at least one bacterial isolate is selected to reduce *Salmonella* and/or *Campylobacter* in the crop of poultry. In one embodiment the at least one bacterial isolate is from Lactic Acid Bacteria. In one embodiment the at least one bacterial isolate is from the genus *Pediococcus* the genus *Lactobacillus* or a combination thereof. In an even more preferred embodiment the at least one bacterial isolate is from the species *Pediococcus pentosaceus*, the species *Pediococcus acidilactici* or the species *Lactobacillus salivarius*, or a combination thereof.

In a specific embodiment of the method the at least one bacterial isolate is selected from the group consisting:

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof; or any combination thereof.

In a specific embodiment of the method the at least one bacterial isolate is *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962.

In a specific embodiment of the method the at least one bacterial isolate is *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320. In a specific embodiment of the method the at least one bacterial isolate is *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178. In a specific embodiment of the method the at least one bacterial isolate is *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179. In a specific embodiment of the method the at least one bacterial isolate is *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177. In a specific embodiment of the method the at least one bacterial isolate is *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

In one aspect the *Salmonella* is *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof. In another aspect the *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*,

Campylobacter upsaliensis, or any combination thereof. In another aspect the *Salmonella* is *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof and the *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof.

In a preferred embodiment the method reduces the level of *Salmonella* in the crop of poultry. In another preferred embodiment the method reduces the level of *Campylobacter* in the crop of poultry. In one embodiment the method reduces the level of *Salmonella* and the level of *Campylobacter* in the crop of poultry.

The administration of the bacterial isolate is preferably oral administration such as via the drinking water. The administration can be a single administration of a specified duration, such as, e.g., one administration of the bacterial isolate lasting the duration of feed withdrawal, such as, e.g., 12-24 hours prior to slaughter, and no further administrations later time point(s). The administration is preferably performed from 5 to 15 hours prior to slaughter, such as from 6 to 14 hours prior to slaughter, such as from 6 to 10 hours prior to slaughter. In another embodiment the administration is performed 7 to 14 hours prior to slaughter, such as from 8 to 14 hours prior to slaughter, such as from 8 to 13 hours prior to slaughter or from 9 to 13 hours prior to slaughter, such as from 8 to 12 hours prior to slaughter, from 9 to 12 hours prior to slaughter, or from 10 to 12 hours prior to slaughter. In another embodiment the administration is performed less than 10 hours prior to feed withdrawal, such as less than 9 hours prior to feed withdrawal, such as less than 8 hours prior to feed withdrawal, such as less than 7 hours prior to feed withdrawal, such as less than 6 hours prior to feed withdrawal, such as less than 5 hours prior to feed withdrawal, such as less than 4 hours prior to feed withdrawal, such as less than 3 hours prior to feed withdrawal, such as less than 2 hours prior to feed withdrawal, such as less than 1 hour prior to feed withdrawal, such as less than 30 minutes prior to feed withdrawal, or such as at the time of feed withdrawal.

In one embodiment the poultry is chicken, broilers, bantams, turkey, duck, geese, guinea fowl, peafowl, quail, dove, pigeon (squab), or pheasant, preferably chicken, turkey or duck.

The invention can result in that *Salmonella* and/or *Campylobacter* levels in the poultry meat or in the poultry crop are reduced by at least one log. In embodiments, the administration reduces *Salmonella* and/or *Campylobacter* levels in the poultry meat or the crop of poultry by at least one log (Log_{10}) compared to the levels of *Salmonella* and/or *Campylobacter* in the absence of this administration. In embodiments, the method reduces *Salmonella* and/or *Campylobacter* levels in the poultry meat or the poultry crop by at least about 0.50, about 1.00, about 1.50, about 2.00, about 2.50 or about 3.00 Log_{10} , including

any and all integers and decimal points in between and above 0.50 (e.g., 0.6, 0.7, 0.8, 0.9). In embodiments, the method decreases *Salmonella* and/or *Campylobacter* levels by at least about 5 fold, 10 fold, 15 fold, 20 fold, 25 fold or 30 fold, including all values in between, less than the amount produced in the absence of the composition, for example, water without the bacterial isolate.

In one embodiment the invention relates to a method for reduction of the levels of *Salmonella* and/or *Campylobacter* in the crop of poultry comprising administering the composition according to the invention to the poultry.

In various aspects, the invention relates to a method for reduction of the levels of *Salmonella* and/or *Campylobacter* in a poultry comprising the step of administering at least one bacterial isolate and at least one bacteriophage to poultry.

In various aspects, the bacterial isolate may be a lactic acid bacteria (LAB). In embodiments, the lactic acid bacteria includes, but is not limited to, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, *Weisella*, or any combination thereof.

The invention further relates to use of the composition according to the invention for reduction of the levels of *Salmonella* and/or *Campylobacter* in poultry such as in poultry meat after slaughter or in the crop of poultry.

Bacterial isolates

The invention relates to bacterial isolates capable of reducing the levels of *Salmonella* and/or *Campylobacter* in poultry. Preferably the bacterial isolates are capable of reducing the levels of *Salmonella* and/or *Campylobacter* in the crop of poultry, e.g., by at least one log (Log_{10}). In embodiments, the bacterial isolate may reduce *Salmonella* and/or *Campylobacter* levels by at least about 0.50, about 1.00, about 1.50, about 2.00, about 2.50 or about 3.00 Log_{10} , including any and all integers and decimal points in between and above 0.50 (e.g., 0.6, 0.7, 0.8, 0.9). In embodiments, the bacterial isolate decreases *Salmonella* and/or *Campylobacter* levels by at least about 5 fold, 10 fold, 15 fold, 20 fold, 25 fold or 30 fold, including all values in between, less than the amount produced in the absence of the composition, for example, water without the bacterial isolate.

In a preferred embodiment the *Salmonella* is *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof. In another preferred embodiment the *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof. In yet another preferred embodiment the *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof and the

Campylobacter is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof.

In a preferred embodiment the bacterial isolate reduces the level of *Salmonella* and/or *Campylobacter* in the crop of poultry, e.g., by at least one log (Log_{10}). Preferably the at least one bacterial isolate is from Lactic Acid Bacteria. In one embodiment the at least one bacterial isolate is from the genus *Pediococcus* the genus *Lactobacillus* or a combination thereof. Preferably the bacterial isolate is from the species *Pediococcus pentosaceus*, the species *Pediococcus acidilactici* or the species *Lactobacillus salivarius*.

In a specific embodiment the bacterial isolate is selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof; or any combination thereof.

The bacterial isolate is in one embodiment *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962.

The bacterial isolate is in one embodiment *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320.

The bacterial isolate is in one embodiment *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178.

The bacterial isolate is in one embodiment *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179.

The bacterial isolate is in one embodiment *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177.

The bacterial isolate is in one embodiment *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

5 In a preferred embodiment the invention relates to a bacterial isolate selected from the group consisting of *Pediococcus acidilactici* 64-C1 deposited as NRRL B-50962, *Lactobacillus salivarius* 64-C2 deposited as NRRL B-67320, *Lactobacillus salivarius* 39B1 deposited as NRRL B-67178, *Pediococcus pentosaceus* 39B2 deposited as NRRL B-67179, *Lactobacillus salivarius* 59B deposited as NRRL B-67177, and *Lactobacillus salivarius* 34B
10 deposited as NRRL B-67180, or a combination thereof.

The bacterial isolates have been deposited with the Agricultural Research Service Culture Collection (NRRL), 1815 North University Street, Peoria, IL 61604, U.S.A., under the above-identified accession numbers.

15 In various embodiments, the bacterial isolate is capable of reducing *Salmonella* and/or *Campylobacter* levels present in the crop of poultry. In embodiments, the bacterial isolate can reduce *Salmonella* and/or *Campylobacter* levels present in the crop of poultry and can survive environmental conditions within the crop. In embodiments, the bacterial isolate can survive at low pH conditions. In embodiments, low pH may be about pH 4 to about pH 7. In embodiments, low pH may be about pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 or 7.0,
20 including any and all integers and decimal points in between and above 4.0 (e.g., 4.6, 4.7, 4.8, 4.9).

Compositions

25 In various aspects, a composition includes at least one bacterial isolate capable of reducing the levels of *Salmonella* and/or *Campylobacter* in poultry, and an agriculturally acceptable excipient. In embodiments, a composition includes at least one bacterial isolate capable of reducing the levels of *Salmonella* and/or *Campylobacter* in poultry.

30 In various aspects, a bacterial isolate may be a lactic acid bacteria (LAB). In embodiments, the lactic acid bacteria includes, but is not limited to, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, *Weisella*, or any combination thereof.

In embodiments, the composition comprises at least one bacterial isolate selected from the group consisting of

35 (a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178;

5 (d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number
10 NRRL B-67180.

or a combination thereof.

In another preferred embodiment the composition comprises at least one bacterial isolate selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number
15 NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

20 (c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;
25

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

30 (f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof;
or any combination thereof.

In various embodiments, the composition includes a bacterial isolate capable of
35 reducing *Salmonella* and/or *Campylobacter* levels. In embodiments, the bacterial isolate reduces *Salmonella* and/or *Campylobacter* levels by at least one log (Log₁₀) compared to the levels of *Salmonella* and/or *Campylobacter* in the absence of the composition. In

embodiments, the bacterial isolate may reduce *Salmonella* and/or *Campylobacter* levels by at least about 0.50, about 1.00, about 1.50, about 2.00, about 2.50 or about 3.00 Log₁₀, including any and all integers and decimal points in between and above 0.50 (e.g., 0.6, 0.7, 0.8, 0.9). In embodiments, the bacterial isolate decreases *Salmonella* and/or *Campylobacter* levels by at least about 5 fold, 10 fold, 15 fold, 20 fold, 25 fold or 30 fold, including all values in between, less than the amount produced in the absence of the composition, for example, water without the composition.

In various embodiments, the composition includes a bacterial isolate capable of reducing *Salmonella* and/or *Campylobacter* levels present in the crop of poultry. In embodiments, the bacterial isolate can reduce *Salmonella* and/or *Campylobacter* levels present in the crop of poultry and can survive environmental conditions within the crop. In embodiments, the bacterial isolate can survive at low pH conditions. In embodiments, low pH may be about pH 4 to about pH 7. In embodiments, low pH may be about pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 or 7.0, including any and all integers and decimal points in between and above 4.0 (e.g., 4.6, 4.7, 4.8, 4.9).

In various aspects, the composition includes at least one bacteriophage capable of reducing the levels of *Salmonella* and/or *Campylobacter* in poultry.

In various embodiments, *Salmonella* is *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof.

In various embodiments, *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof.

In various aspects, a composition includes at least one anti-*Salmonella* bacterial isolate. In various aspects, a composition includes at least one anti-*Campylobacter* bacterial isolate. In various aspects, a composition includes at least one anti-*Salmonella* bacterial isolate and at least one anti-*Campylobacter* bacterial isolate.

In a specific embodiment the composition comprises at least two bacterial isolates (such as at least three, such as at least four, such as at least five, such as at least six) selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

5 (e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius*
10 34B having the deposit accession number NRRL B-67180 or a mutant thereof;
or any combination thereof.

In another specific embodiment the composition comprises at least two bacterial isolates (such as at least three, such as at least four, such as at least five, such as at least six) selected from the group consisting of

15 (a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number
20 NRRL B-67178;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; and

25 (f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

In a particularly preferred embodiment the composition comprises at least

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962 and

30 (b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320.

In another particularly preferred embodiment the composition comprises at least

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof
35 and

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof.

5 Selection Methods

Various aspects relate to methods of selecting bacterial isolates for reducing *Salmonella* and/or *Campylobacter* levels in poultry such as chickens.

In various aspects, a selection method for identifying a bacterial isolate capable of reducing *Salmonella* and/or *Campylobacter* levels is provided. In embodiments, a bacterial isolate is screened using an *in vitro* method. In embodiments, a bacterial isolate may be identified using selective agar plating. In embodiments, a bacterial isolate may be combined with *Salmonella* or *Campylobacter* and plated onto an agar plate to identify growth of the *Salmonella* or *Campylobacter* in the presence of the bacterial isolate compared to the growth of *Salmonella* or *Campylobacter* in the absence of a bacterial isolate.

In embodiments, a bacterial isolate capable of reducing *Salmonella* and/or *Campylobacter* levels may be further screened using an *in vivo* method. In embodiments, a bacterial isolate may be administered to a chicken and the number of *Salmonella* or *Campylobacter* recovered from the chicken may be determined and compared to the number recovered from a chicken not administered a bacterial isolate.

In various embodiments, a bacterial isolate may be capable of surviving various conditions, for example, various environments within the gastrointestinal tract of an animal. A bacterial isolate that survives various conditions is a bacterial isolate that maintains activity against and is capable of reducing levels of *Salmonella* and/or *Campylobacter* under such conditions. In embodiments, a bacterial isolate may be capable of surviving various pH ranges. In some embodiments, a bacterial isolate survives a pH range of pH 4.5 to pH 8.0. In embodiments, a bacterial isolate is capable of reducing *Salmonella* and/or *Campylobacter* levels at a pH range of pH 4.5 to pH 8.4. In embodiments, a bacterial isolate survives a pH of about 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0.

In various embodiments, a bacterial isolate may be administered to a chicken prior to or at the time of feed withdrawal. In embodiments, the feed withdrawal period may be about 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, or 24 hours. In some embodiments, the feed withdrawal period may be about 8 hours or about 12 hours.

In embodiments, a bacterial isolate may be capable of reducing *Salmonella* and/or *Campylobacter* in the gastrointestinal tract of an animal. In embodiments, a bacterial isolate may be capable of reducing *Salmonella* and/or *Campylobacter* in the crop of poultry. The

crop is a part of the esophagus where the initial stages of digestion occur. In one embodiment the poultry is chicken, broilers, bantams, turkey, duck, geese, guinea fowl, peafowl, quail, dove, pigeon (squab), or pheasant, preferably chicken, turkey or duck.

In various embodiments, a bacterial isolate may reduce *Salmonella* and/or *Campylobacter* levels by at least one log (Log_{10}). In embodiments, a bacterial isolate may reduce *Salmonella* and/or *Campylobacter* levels by at least about 0.50, about 1.00, about 1.50, about 2.00, about 2.50 or about 3.00 Log_{10} , including any and all integers in between.

In various embodiments, a bacterial isolate may be selected as specific for a single *Salmonella* and/or *Campylobacter* species. In embodiments, a single *Salmonella* and/or *Campylobacter* species may have been identified at a particular poultry farm, poultry house or poultry company. In embodiments, a bacterial isolate may be selected to specifically reduce the pre-identified *Salmonella* and/or *Campylobacter* species.

PREFERRED EMBODIMENTS

Preferred embodiments of the invention are disclosed in the set of items herein below.

1. A method for reducing the level of *Salmonella* and/or *Campylobacter* in poultry meat comprising the step of administering at least one bacterial isolate to the poultry less than 24 hours prior to slaughter preferably at the time of feed withdrawal or during the feed withdrawal period.

2. The method of item 1, wherein the at least one bacterial isolate is selected to reduce *Salmonella* and/or *Campylobacter* in the crop of poultry.

3. The method of item 1 or 2, wherein the at least one bacterial isolate is from the species *Pediococcus pentosaceus* or the species *Lactobacillus salivarius*, or a combination thereof.

4. The method of any of items 1-3, wherein the at least one bacterial isolate is selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof; or any combination thereof.

5. The method of any of items 1-3, wherein the at least one bacterial isolate is *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962.

6. The method of any of items 1-3, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320.

7. The method of any of items 1-3, wherein the at least one bacterial isolate is from the species *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178.

8. The method of any of items 1-3, wherein the at least one bacterial isolate is *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179.

9. The method of any of items 1-3, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177.

10. The method of any of items 1-3, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

11. The method of any of items 1-10, wherein the *Salmonella* is *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof.
- 5 12. The method of any of items 1-11, wherein the *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof.
- 10 13. The method of any of items 1-12, wherein the method reduces the level of *Salmonella* in the crop of poultry.
14. The method of any of items 1-13, wherein the method reduces the level of *Campylobacter* in the crop of poultry.
- 15 15. The method of any of items 1-14, wherein the administration is via the drinking water.
16. The method of any of items 1-15, wherein the administration is performed from 5 to 15 hours prior to slaughter, such as from 6 to 14 hours prior to slaughter, such as from 6 to 10 hours prior to slaughter.
- 20 17. The method of any of items 1-16, wherein the administration is performed less than 10 hours prior to feed withdrawal, such as less than 9 hours prior to feed withdrawal, such as less than 8 hours prior to feed withdrawal, such as less than 7 hours prior to feed withdrawal, such as less than 6 hours prior to feed withdrawal, such as less than 5 hours prior to feed withdrawal, such as less than 4 hours prior to feed withdrawal, such as less than 3 hours prior to feed withdrawal, such as less than 2 hours prior to feed withdrawal, such as less than 1 hour prior to feed withdrawal, such as less than 30 minutes prior to feed withdrawal, or such as at the time of feed withdrawal.
- 25 18. The method of any of items 1-17, wherein the poultry is chicken, broilers, bantams, turkey, duck, geese, guinea fowl, peafowl, quail, dove, pigeon (squab), or pheasant, preferably chicken, turkey or duck.
- 30 19. The method of any of items 1-18, wherein said *Salmonella* and/or *Campylobacter* levels are reduced by at least one log compared to the levels of *Salmonella* and/or *Campylobacter* in the absence of the bacterial isolate.
- 35

20. The method of any of items 1-19, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and/or SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and/or SEQ ID NO: 6).

21. The method of any of items 1-20, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1).

22. The method of any of items 1-21, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 2 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 2 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 2).

23. The method of any of items 1-22, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 3 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 3 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 3).

24. The method of any of items 1-23, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 4 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 4 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 4).

25. The method of any of items 1-24, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 5 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 5 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 5).

26. The method of any of items 1-25, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 6 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 6).

27. A bacterial isolate reducing the level of *Salmonella* and/or *Campylobacter* in the crop of poultry.

28. The bacterial isolate of item 27, wherein the bacterial isolate is from the species *Pediococcus pentosaceus* or a *Lactobacillus salivarius*.

29. The bacterial isolate of item 27 or 28, wherein the bacterial isolate is selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof; or any combination thereof.

30. The bacterial isolate of any of items 27-29, wherein the bacterial isolate is *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962.

31. The bacterial isolate of any of items 27-29, wherein the bacterial isolate is *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320.

32. The bacterial isolate of any of items 27-29, wherein the bacterial isolate is from the species *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178.

5 33. The bacterial isolate of any of items 27-29, wherein the bacterial isolate is *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179.

34. The bacterial isolate of any of items 27-29, wherein the bacterial isolate is
10 *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177.

35. The bacterial isolate of any of items 27-32, wherein the bacterial isolate is *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

15 36. The bacterial isolate of any of items 27-35, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and/or SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5
20 and/or SEQ ID NO: 6).

37. The bacterial isolate of any of items 27-36, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5%
25 sequence identity to SEQ ID NO: 1).

38. The bacterial isolate of any of items 27-37, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 2 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 2 (such as at least 99% or 99.5%
30 sequence identity to SEQ ID NO: 2).

39. The bacterial isolate of any of items 27-38, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 3 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 3 (such as at least 99% or 99.5%
35 sequence identity to SEQ ID NO: 3).

40. The bacterial isolate of any of items 27-39, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 4 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 4 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 4).

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41. The bacterial isolate of any of items 27-40, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 5 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 5 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 5).

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42. The bacterial isolate of any of items 27-41, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 6 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 6).

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43. A composition comprising a bacterial isolate of any of items 27-42 and an agriculturally acceptable excipient.

44. The composition of item 43 comprising at least two bacterial isolates (such as at least three, such as at least four, such as at least five, such as at least six) selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

25 (b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

30 (d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

35 (e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof; or any combination thereof.

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45. The composition of item 43 comprising at least two bacterial isolates selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962;

10 (b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178;

15 (d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

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46. The composition of item 45 comprising at least three bacterial isolates.

47. The composition of item 45 comprising at least four bacterial isolates.

25 48. The composition of item 45 comprising at least five bacterial isolates.

49. The composition of item 45 comprising at least six bacterial isolates.

30 50. A method for selecting at least one bacterial isolate for reducing *Salmonella* and/or *Campylobacter* levels in poultry comprising the steps of:

i) identifying a bacterial isolate capable of reducing *Salmonella* and/or *Campylobacter* levels *in vitro*;

ii) testing said bacterial isolate *in vivo*; and

35 iii) selecting a bacterial isolate of reducing *Salmonella* and/or *Campylobacter* levels *in vivo*.

51. The method of item 50, wherein the at least one bacterial isolate is from the species *Pediococcus pentosaceus*, the species *Pediococcus acidilactici* or the species *Lactobacillus salivarius*, or a combination thereof.

5 52. The method of item 50 or 51, wherein the *Salmonella* is *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof.

10 53. The method of any of items 50-52, wherein the *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof.

54. The method of any of items 50-53, wherein said *Salmonella* and/or *Campylobacter* levels are reduced by at least one log.

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FURTHER PREFERRED EMBODIMENTS

Further preferred embodiments of the invention are disclosed in the set of items herein below.

20 1. A method for reducing the level of *Salmonella* and/or *Campylobacter* in poultry meat comprising the step of administering at least one bacterial isolate to the poultry less than 24 hours prior to slaughter preferably at the time of feed withdrawal or during the feed withdrawal period.

25 2. The method of item 1 for reducing the level of *Salmonella* and *Campylobacter* in poultry meat.

3. The method of item 1 or 2, wherein the at least one bacterial isolate is selected to reduce *Salmonella* and/or *Campylobacter* in the crop of poultry.

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4. A method for reducing the level of *Salmonella* and/or *Campylobacter* in poultry meat comprising the step of administering at least one bacterial isolate to the poultry less than 24 hours prior to slaughter preferably at the time of feed withdrawal or during the feed withdrawal period, wherein the at least one bacterial isolate is selected to reduce *Salmonella*
35 and/or *Campylobacter* in the crop of poultry.

5. The method of item 4 for reducing the level of *Salmonella* and *Campylobacter* in poultry meat.
6. The method of any of items 1-5, wherein the at least one bacterial isolate is from Lactic Acid Bacteria.
7. The method of any of items 1-6, wherein the at least one bacterial isolate is from the genus *Pediococcus* or the genus *Lactobacillus* or a combination thereof.
8. The method of any of items 1-7, wherein the at least one bacterial isolate is from the species *Pediococcus pentosaceus*, the species *Pediococcus acidilactici* or the species *Lactobacillus salivarius*, or a combination thereof.
9. The method of any of items 1-8, wherein the at least one bacterial isolate is selected from the group consisting of
- (a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;
 - (b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;
 - (c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;
 - (d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;
 - (e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and
 - (f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof;
- or any combination thereof.

10. The method of any of items 1-8, wherein the at least one bacterial isolate is *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962.

11. The method of any of items 1-8, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320.

12. The method of any of items 1-8, wherein the at least one bacterial isolate is from the species *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178.

13. The method of any of items 1-8, wherein the at least one bacterial isolate is *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179.

14. The method of any of items 1-8, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177.

15. The method of any of items 1-8, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

16. The method of any of items 1-15, wherein the *Salmonella* is *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof.

17. The method of any of items 1-16, wherein the *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof.

18. The method of any of items 1-17, wherein the method reduces the level of *Salmonella* in the crop of poultry.

19. The method of any of items 1-17, wherein the method reduces the level of *Campylobacter* in the crop of poultry.

20. The method of any of items 1-17, wherein the method reduces the level of *Salmonella* and *Campylobacter* in the crop of poultry.

21. The method of any of items 1-20, wherein the administration is via the drinking water.

22. The method of any of items 1-21, wherein the administration is in the form of a single administration.

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23. The method of any of items 1-21, wherein the at least one bacterial isolate is administered to the poultry one time only.

24. The method of any of items 1-23, wherein the administration is performed from 5 to 10 15 hours prior to slaughter, such as from 6 to 14 hours prior to slaughter, such as from 6 to 10 hours prior to slaughter.

25. The method of any of items 1-23, wherein the administration is performed from 7 to 14 hours prior to slaughter, such as from 8 to 14 hours prior to slaughter, such as from 8 to 15 13 hours prior to slaughter or from 9 to 13 hours prior to slaughter, such as from 8 to 12 hours prior to slaughter, from 9 to 12 hours prior to slaughter, or from 10 to 12 hours prior to slaughter.

26. The method of any of items 1-25, wherein the administration is performed less than 20 10 hours prior to feed withdrawal, such as less than 9 hours prior to feed withdrawal, such as less than 8 hours prior to feed withdrawal, such as less than 7 hours prior to feed withdrawal, such as less than 6 hours prior to feed withdrawal, such as less than 5 hours prior to feed withdrawal, such as less than 4 hours prior to feed withdrawal, such as less than 3 hours prior to feed withdrawal, such as less than 2 hours prior to feed withdrawal, such as less 25 than 1 hour prior to feed withdrawal, such as less than 30 minutes prior to feed withdrawal, or such as at the time of feed withdrawal.

27. The method of any of items 1-26, wherein the poultry is chicken, broilers, bantams, turkey, duck, geese, guinea fowl, peafowl, quail, dove, pigeon (squab), or pheasant, 30 preferably chicken, turkey or duck.

28. The method of any of items 1-27, wherein said *Salmonella* and/or *Campylobacter* levels are reduced by at least one log compared to the levels of *Salmonella* and/or *Campylobacter* in the absence of the bacterial isolate.

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29. The method of any of items 1-28, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4,

SEQ ID NO: 5 and/or SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and/or SEQ ID NO: 6).

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30. The method of any of items 1-29, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1).

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31. The method of any of items 1-29, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 2 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 2 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 2).

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32. The method of any of items 1-29, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 3 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 3 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 3).

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33. The method of any of items 1-29, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 4 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 4 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 4).

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34. The method of any of items 1-29, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 5 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 5 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 5).

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35. The method of any of items 1-29, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 6 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 6).

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36. A bacterial isolate reducing the level of *Salmonella* and/or *Campylobacter* in the crop of poultry.

37. A bacterial isolate of item 36, which is reducing the level of *Salmonella* and *Campylobacter* in the crop of poultry.

5 38. The bacterial isolate of item 36 or 37, wherein the at least one bacterial isolate is from Lactic Acid Bacteria.

39. The bacterial isolate of item 36 or 37, wherein the at least one bacterial isolate is from the genus *Pediococcus* or the genus *Lactobacillus* or a combination thereof.

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40. The bacterial isolate of item 36 or 37, wherein the bacterial isolate is from the species *Pediococcus pentosaceus*, the species *Pediococcus acidilactici* or the species *Lactobacillus salivarius*, or a combination thereof.

15 41. The bacterial isolate of any of items 36-40, wherein the bacterial isolate is selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

20 (b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

25 (d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

30 (e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof;

35 or any combination thereof.

42. The bacterial isolate of any of items 36-41, wherein the bacterial isolate is *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962.

43. The bacterial isolate of any of items 36-41, wherein the bacterial isolate is *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320.

44. The bacterial isolate of any of items 36-41, wherein the bacterial isolate is from the species *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178.

45. The bacterial isolate of any of items 36-41, wherein the bacterial isolate is *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179.

46. The bacterial isolate of any of items 36-41, wherein the bacterial isolate is *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177.

47. The bacterial isolate of any of items 36-41, wherein the bacterial isolate is *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

48. The bacterial isolate of any of items 36-41, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and/or SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and/or SEQ ID NO: 6).

49. The bacterial isolate of any of items 36-41 or 48, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1).

50. The bacterial isolate of any of items 36-41 or 48, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 2 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 2 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 2).

51. The bacterial isolate of any of items 36-41 or 48, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 3 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 3 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 3).

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52. The bacterial isolate of any of items 36-41 or 48, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 4 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 4 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 4).

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53. The bacterial isolate of any of items 36-41 or 48, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 5 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 5 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 5).

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54. The bacterial isolate of any of items 36-41 or 48, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 6 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 6).

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55. A composition comprising a bacterial isolate of any of items 36-54 and an agriculturally acceptable excipient.

56. A composition comprising a bacterial isolate of any of items 36-54 and an agriculturally acceptable excipient which is selected from the group consisting of: Dry skim milk powder, inulin, fructo-oligosaccharides, soy protein, pea protein, yeast extract, sodium ascorbate, vitamin C, citric acid, mannan-oligosaccharides, dipotassium phosphate, phosphate buffers, lactose, trehalose and casein.

30 57. The composition of item 55 or 56 which does not comprise a monosaccharide.

58. The composition of item 55 or 56 which does not comprise a disaccharide.

35 59. The composition of item 55 or 56 which does not comprise a monosaccharide or a disaccharide.

60. The composition of any of items 55-59 comprising at least two bacterial isolates (such as at least three, such as at least four, such as at least five, such as at least six) selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof; or any combination thereof.

61. The composition of any of items 55-59 comprising at least two bacterial isolates selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

62. The composition of any of items 55-59 comprising at least three bacterial isolates.

63. The composition of any of items 55-59 comprising at least four bacterial isolates.

64. The composition of any of items 55-59 comprising at least five bacterial isolates.

65. The composition of any of items 55-59 comprising at least six bacterial isolates.

66. A method for selecting at least one bacterial isolate for reducing *Salmonella* and/or *Campylobacter* levels in poultry comprising the steps of:

i) identifying a bacterial isolate capable of reducing *Salmonella* and/or *Campylobacter* levels *in vitro*;

ii) testing said bacterial isolate *in vivo*; and

iii) selecting a bacterial isolate of reducing *Salmonella* and/or *Campylobacter* levels *in vivo*.

67. The method of item 66, wherein the at least one bacterial isolate is from Lactic Acid Bacteria.

68. The method of item 66, wherein the at least one bacterial isolate is from the genus *Pediococcus* or the genus *Lactobacillus* or a combination thereof.

69. The method of item 68, wherein the at least one bacterial isolate is from the species *Pediococcus pentosaceus*, the species *Pediococcus acidilactici* or the species *Lactobacillus salivarius*, or a combination thereof.

70. The method of any of items 66-69, wherein the *Salmonella* is *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof.

71. The method of any of items 66-69, wherein the *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof.

72. The method of any of items 66-71, wherein said *Salmonella* and/or *Campylobacter* levels are reduced by at least one log.

73. A bacterial isolate for use in the treatment of poultry for reducing the level of *Salmonella* and/or *Campylobacter* in poultry meat, wherein the at least one bacterial isolate is administered to the poultry less than 24 hours prior to slaughter preferably at the time of feed withdrawal or during the feed withdrawal period.

74. The bacterial isolate of item 73 for reducing the level of *Salmonella* and *Campylobacter* in poultry meat.

75. The bacterial isolate of item 73 or 74, wherein the treatment reduces *Salmonella* and/or *Campylobacter* in the crop of poultry.

76. A bacterial isolate for use in the treatment of poultry for reducing the level of *Salmonella* and/or *Campylobacter* in poultry meat comprising the step of administering at least one bacterial isolate to the poultry less than 24 hours prior to slaughter preferably at the time of feed withdrawal or during the feed withdrawal period, wherein the at least one bacterial isolate is selected to reduce *Salmonella* and/or *Campylobacter* in the crop of poultry.

77. The bacterial isolate of item 76 for reducing the level of *Salmonella* and *Campylobacter* in poultry meat.

78. The bacterial isolate of any of items 73-77, wherein the at least one bacterial isolate is from Lactic Acid Bacteria.

79. The bacterial isolate of any of items 73-77, wherein the at least one bacterial isolate is from the genus *Pediococcus* or the genus *Lactobacillus* or a combination thereof.

80. The bacterial isolate of any of items 73-77, wherein the at least one bacterial isolate is from the species *Pediococcus pentosaceus*, the species *Pediococcus acidilactici* or the species *Lactobacillus salivarius*, or a combination thereof.

81. The bacterial isolate of any of items 73-77, wherein the at least one bacterial isolate is selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof;

or any combination thereof.

82. The bacterial isolate of any of items 73-81, wherein the at least one bacterial isolate is *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962.

83. The bacterial isolate of any of items 73-81, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320.

84. The bacterial isolate of any of items 73-81, wherein the at least one bacterial isolate is from the species *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178.

85. The bacterial isolate of any of items 73-81, wherein the at least one bacterial isolate is *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179.

86. The bacterial isolate of any of items 73-81, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177.

87. The bacterial isolate of any of items 73-81, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

88. The bacterial isolate of any of items 73-87, wherein the *Salmonella* is *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof.

89. The bacterial isolate of any of items 73-87, wherein the *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof.

90. The bacterial isolate of any of items 73-88, wherein the bacterial isolate reduces the level of *Salmonella* in the crop of poultry.

91. The bacterial isolate of any of items 73-87 or 89, wherein the bacterial isolate reduces the level of *Campylobacter* in the crop of poultry.

92. The bacterial isolate of any of items 73-91, wherein the bacterial isolate reduces the level of *Salmonella* and *Campylobacter* in the crop of poultry.

93. The bacterial isolate of any of items 73-92, wherein the administration is via the drinking water.

94. The bacterial isolate of any of items 73-93, wherein the administration is in the form of a single administration.

95. The bacterial isolate of any of items 73-93, wherein the at least one bacterial isolate is administered to the poultry one time only.

96. The bacterial isolate of any of items 73-95, wherein the administration is performed from 5 to 15 hours prior to slaughter, such as from 6 to 14 hours prior to slaughter, such as from 6 to 10 hours prior to slaughter.

97. The bacterial isolate of any of items 73-95, wherein the administration is performed from 7 to 14 hours prior to slaughter, such as from 8 to 14 hours prior to slaughter, such as from 8 to 13 hours prior to slaughter or from 9 to 13 hours prior to slaughter, such as from 8 to 12 hours prior to slaughter, from 9 to 12 hours prior to slaughter, or from 10 to 12 hours prior to slaughter.

98. The bacterial isolate of any of items 73-97, wherein the administration is performed less than 10 hours prior to feed withdrawal, such as less than 9 hours prior to feed withdrawal, such as less than 8 hours prior to feed withdrawal, such as less than 7 hours prior to feed withdrawal, such as less than 6 hours prior to feed withdrawal, such as less than 5 hours prior to feed withdrawal, such as less than 4 hours prior to feed withdrawal, such as less than 3 hours prior to feed withdrawal, such as less than 2 hours prior to feed withdrawal, such as less than 1 hour prior to feed withdrawal, such as less than 30 minutes prior to feed withdrawal, or such as at the time of feed withdrawal.

99. The bacterial isolate of any of items 73-98, wherein the poultry is chicken, broilers, bantams, turkey, duck, geese, guinea fowl, peafowl, quail, dove, pigeon (squab), or pheasant, preferably chicken, turkey or duck.

100. The bacterial isolate of any of items 73-99, wherein said *Salmonella* and/or *Campylobacter* levels are reduced by at least one log compared to the levels of *Salmonella* and/or *Campylobacter* in the absence of the bacterial isolate.

101. The bacterial isolate of any of items 73-100, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and/or SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and/or SEQ ID NO: 6).

102. The bacterial isolate of any of items 73-101, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1).

103. The bacterial isolate of any of items 73-101, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 2 or comprising a 16S rDNA sequence

having at least 98% sequence identity to SEQ ID NO: 2 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 2).

104. The bacterial isolate of any of items 73-101, wherein the at least one bacterial isolate
5 has a 16S rDNA sequence comprising SEQ ID NO: 3 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 3 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 3).

105. The bacterial isolate of any of items 73-101, wherein the at least one bacterial isolate
10 has a 16S rDNA sequence comprising SEQ ID NO: 4 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 4 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 4).

106. The bacterial isolate of any of items 73-101, wherein the at least one bacterial isolate
15 has a 16S rDNA sequence comprising SEQ ID NO: 5 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 5 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 5).

107. The bacterial isolate of any of items 73-101, wherein the at least one bacterial isolate
20 has a 16S rDNA sequence comprising SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 6 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 6).

108. Use of at least one bacterial isolate in the manufacture of a medicament for reducing
25 the level of *Salmonella* and/or *Campylobacter* in poultry wherein the at least one bacterial isolate is administered to the poultry less than 24 hours prior to slaughter, preferably at the time of feed withdrawal or during the feed withdrawal period.

109. Use of at least one bacterial isolate for reducing the level of *Salmonella* and/or
30 *Campylobacter* in poultry meat comprising the step of administering at least one bacterial isolate to the poultry less than 24 hours prior to slaughter preferably at the time of feed withdrawal or during the feed withdrawal period.

110. The use of item 108 or 109 for reducing the level of *Salmonella* and *Campylobacter*
35 in poultry meat.

111. The use of any of items 108-110, wherein the at least one bacterial isolate is selected to reduce *Salmonella* and/or *Campylobacter* in the crop of poultry.

112. Use of at least one bacterial isolate for reducing the level of *Salmonella* and/or *Campylobacter* in poultry meat comprising the step of administering at least one bacterial isolate to the poultry less than 24 hours prior to slaughter preferably at the time of feed withdrawal or during the feed withdrawal period, wherein the at least one bacterial isolate is selected to reduce *Salmonella* and/or *Campylobacter* in the crop of poultry.

113. The use of item 112 for reducing the level of *Salmonella* and *Campylobacter* in poultry meat.

114. The use of any of items 108-113, wherein the at least one bacterial isolate is from Lactic Acid Bacteria.

115. The use of any of items 108-113, wherein the at least one bacterial isolate is from the genus *Pediococcus* or the genus *Lactobacillus* or a combination thereof.

116. The use of any of items 108-113, wherein the at least one bacterial isolate is from the species *Pediococcus pentosaceus*, the species *Pediococcus acidilactici* or the species *Lactobacillus salivarius*, or a combination thereof.

117. The use of any of items 108-113, wherein the at least one bacterial isolate is selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof; or any combination thereof.

118. The use of any of items 108-117, wherein the at least one bacterial isolate is *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962.

119. The use of any of items 108-117, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320.

120. The use of any of items 108-117, wherein the at least one bacterial isolate is from the species *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178.

121. The use of any of items 108-117, wherein the at least one bacterial isolate is *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179.

122. The use of any of items 108-117, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177.

123. The use of any of items 108-117, wherein the at least one bacterial isolate is *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180.

124. The use of any of items 108-123, wherein the *Salmonella* is *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof.

125. The use of any of items 108-123, wherein the *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof.

126. The use of any of items 108-124, wherein the use reduces the level of *Salmonella* in the crop of poultry.

5 127. The use of any of items 108-123 or 125, wherein the use reduces the level of *Campylobacter* in the crop of poultry.

128. The use of any of items 108-127, wherein the use reduces the level of *Salmonella* and *Campylobacter* in the crop of poultry.

10 129. The use of any of items 108-128, wherein the administration is via the drinking water.

130. The use of any of items 108-129, wherein the administration is in the form of a single administration.

15 131. The use of any of items 108-129, wherein the at least one bacterial isolate is administered to the poultry one time only.

132. The use of any of items 108-131, wherein the administration is performed from 5 to 15 hours prior to slaughter, such as from 6 to 14 hours prior to slaughter, such as from 6 to 20 10 hours prior to slaughter.

133. The use of any of items 108-131, wherein the administration is performed from 7 to 14 hours prior to slaughter, such as from 8 to 14 hours prior to slaughter, such as from 8 to 13 hours prior to slaughter or from 9 to 13 hours prior to slaughter, such as from 8 to 12 25 hours prior to slaughter, from 9 to 12 hours prior to slaughter, or from 10 to 12 hours prior to slaughter.

134. The use of any of items 108-133, wherein the administration is performed less than 10 hours prior to feed withdrawal, such as less than 9 hours prior to feed withdrawal, such as 30 less than 8 hours prior to feed withdrawal, such as less than 7 hours prior to feed withdrawal, such as less than 6 hours prior to feed withdrawal, such as less than 5 hours prior to feed withdrawal, such as less than 4 hours prior to feed withdrawal, such as less than 3 hours prior to feed withdrawal, such as less than 2 hours prior to feed withdrawal, such as less than 1 hour prior to feed withdrawal, such as less than 30 minutes prior to feed withdrawal, 35 or such as at the time of feed withdrawal.

135. The use of any of items 108-134, wherein the poultry is chicken, broilers, bantams, turkey, duck, geese, guinea fowl, peafowl, quail, dove, pigeon (squab), or pheasant, preferably chicken, turkey or duck.

- 5 136. The use of any of items 108-135, wherein said *Salmonella* and/or *Campylobacter* levels are reduced by at least one log compared to the levels of *Salmonella* and/or *Campylobacter* in the absence of the bacterial isolate.

10 137. The use of any of items 108-136, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and/or SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and/or SEQ ID NO: 6).

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138. The use of any of items 108-137, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 1 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 1 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 1).

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139. The use of any of items 108-137, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 2 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 2 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 2).

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140. The use of any of items 108-137, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 3 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 3 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 3).

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141. The use of any of items 108-137, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 4 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 4 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 4).

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142. The use of any of items 108-137, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 5 or comprising a 16S rDNA sequence having at

least 98% sequence identity to SEQ ID NO: 5 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 5).

143. The use of any of items 108-137, wherein the at least one bacterial isolate has a 16S rDNA sequence comprising SEQ ID NO: 6 or comprising a 16S rDNA sequence having at least 98% sequence identity to SEQ ID NO: 6 (such as at least 99% or 99.5% sequence identity to SEQ ID NO: 6).

EXAMPLES

10 Identification, characterization and deposit of the biological material

The following biological materials were deposited under the terms of the Budapest Treaty at Agricultural Research Service Culture Collection (NRRL), 1815 North University Street, Peoria, IL 61604, U.S.A., and given the following accession numbers:

15 Deposit of Biological Material

Identification	Accession Number	Date of Deposit
<i>Pediococcus acidilactici</i> 64-C1	NRRL Deposit Number B-50962	April 1 2014
<i>Lactobacillus salivarius</i> 64-C2	NRRL Deposit Number B- 67320	September 23 2016
<i>Lactobacillus salivarius</i> 39B1	NRRL Deposit Number B-67178	December 22 2015
<i>Pediococcus pentosaceus</i> 39B2	NRRL Deposit Number B-67179	December 22 2015
<i>Lactobacillus salivarius</i> 59B	NRRL Deposit Number B-67177	December 22 2015
<i>Lactobacillus salivarius</i> 34B	NRRL Deposit Number B-67180	December 22 2015

- The strains have been deposited under conditions that assure that access to the culture will be available during the pendency of this patent application to one determined by foreign patent laws to be entitled thereto. The deposits represent a substantially pure culture of the deposited strain. The deposits are available as required by foreign patent laws in countries wherein counterparts of the subject application or its progeny are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

25 Description of the biological material

Pediococcus acidilactici 64C1 was isolated by Pacific Vet Group from a poultry sample in the US in 2011. The strain was identified as *Pediococcus acidilactici* based on

16S rDNA sequencing. *Lactobacillus salivarius* 64-C2 was isolated by Pacific Vet Group from a poultry sample in the US in 2011. The strain was identified as *Lactobacillus salivarius* based on 16S rDNA sequencing. *Lactobacillus salivarius* 39B1 was isolated by Pacific Vet Group from a poultry sample in the US in 2011. The strain was identified as *Lactobacillus salivarius* based on 16S rDNA sequencing. *Pediococcus pentosaceus* 39B2 was isolated by Pacific Vet Group from a poultry sample in the US in 2011. The strain was identified as *Pediococcus pentosaceus* based on 16S rDNA sequencing. *Lactobacillus salivarius* 59B was isolated by Pacific Vet Group from a poultry sample in the US in 2011. The strain was identified as *Lactobacillus salivarius* based on 16S rDNA sequencing. *Lactobacillus salivarius* 34B was isolated by Pacific Vet Group from a poultry sample in the US in 2011. The strain was identified as *Lactobacillus salivarius* based on 16S rDNA sequencing.

Example 1: Probiotic Bacterial Isolate Screening

Candidate bacterial isolates were screened as illustrated in FIG. 1. Bacterial isolates were selected from either a library of isolates previously screened for probiotic activity or novel strains isolated from the crop of market age broiler chickens. Bacterial isolates were blind passaged and tested in an *in vitro* crop assay. Bacterial candidates were tested for the ability to reduce *Salmonella* or *Campylobacter* by at least 1 log. Bacterial candidates not possessing this ability were discarded.

In Vitro Crop Assay

A 600 μ l sample of a bacterial candidate (at 1×10^6) was incubated with 500 μ l *Salmonella enteritidis* (at 10^5 from an overnight culture) or 500 μ l of *Campylobacter jejuni* (10^5 from an overnight culture), 4.5 ml saline and 1.25 g of a standard corn-soy based broiler chicken starter feed. The assay was performed in replicates of 5. The samples were serially diluted and plated on agar plates with antibiotics. The bacterial candidates were incubated for 6 hours at 42°C and cfu/ml ($\log_{10}$) determined (cf. FIG. 2).

In vitro Crop Assay Against Salmonella Enteritidis

Lactic acid bacteria (LAB) isolates were tested in an *in vitro* crop assay as described above and in (FIG. 2) and tested against *Salmonella enteritidis*. LAB isolates and combinations of LAB isolates were incubated with *Salmonella enteritidis* for 6 hours at 42°C in the crop assay. After incubation, a sample was taken for enumeration. Samples were serially diluted and plated on brilliant green agar containing novobiocin and nalidixic acid. Plates were incubated at least 18 hours at 37°C before reading (cf. FIG. 3).

In vitro Crop Assay Against Campylobacter jejuni

Further lactic acid bacteria (LAB) isolates were selected and tested against *Campylobacter jejuni* in the crop assay described above and in (FIG. 2). LAB isolates and combinations of LAB isolates were incubated with *Campylobacter* for 6 hours at 42°C in buffered saline at pH 6.2 (FIG. 4) or pH 7.2 (saline, FIG. 5) in the crop assay. After incubation, a sample was taken for enumeration. Samples were serially diluted and plated on tryptic soy agar with sheep blood. Plates were incubated at least 18 hours at 37°C before reading.

In vitro Test Against Common Poultry Pathogens.

LAB isolates (64-C1 and 64-C2) were incubated on agar plates with *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, *Clostridium perfringens* and *Escherichia coli* and the zone of inhibition determined Figure 6. Briefly, the LAB isolates were grown in de Man, Rogosa and Sharpe (MRS) broth overnight while being incubated at 37°C. A sterile loop was used to transfer the LAB onto an MRS plate so that a single dense colony will grow on the center of a plate or bi-plate. This plate was incubated for 48 hours at 37°C. During incubation an overnight culture of the pathogen of interest was incubated in appropriate media at 37°C. 100 microliters of the pathogen culture was added to 10ml of tryptic soy soft agar held at 45°C. The tryptic soy soft agar was poured over the MRS plate containing the LAB isolate and incubated overnight at 37°C.

Example 2: In Vivo Testing

Experiment 1

LAB candidates were provided as probiotic formulations to eight week old broiler chickens during an eight (8) hour feed withdrawal period in a small scale single replicate trial (n=12 per group. All LAB formulations were administered via the drinking water at 10⁸ cfu/ml. Chickens were experimentally infected with *Salmonella enteritidis* the day prior to treatment. The crop of the chickens was removed and tested for the presence of *Salmonella enteritidis*. Briefly, the chickens were humanely euthanized. The crop was aseptically removed and macerated. Sterile saline was added (4 times the weight of the sample). A sample of the crop/saline solution was taken and serially diluted then directly plated onto brilliant green agar (BGA). To the remainder of the crop/saline solution tetrathionate broth was added to double the initial volume. Both the BGA plates and crop solutions were incubated overnight at 37°C. BGA plates were read and data were recorded. A sample from the crop solution was then struck onto BGA plates and incubated at 37°C overnight for determination of percent of chickens with *Salmonella*. FIG. 7 illustrates the percent of chickens from which *Salmonella enteritidis* was recovered. FIG. 8 illustrates the number of colony forming units

(cfu) per gram of cecal content (Log_{10}) of *Salmonella enteritidis* recovered. In this experiment several LAB formulations (JH-21, JH-72, 64C, JH-63, and PHL-34B) were compared to a negative control (Con) that was not treated. In this experiment only on treatment group 64C met the criteria of at least 1 log_{10} cfu/g reduction of *Salmonella* in the crop. Similarly, group 64C was also the only group to reduce incidence as reported as percentage *Salmonella* positive broiler chickens. This group was selected for further study in multiple replicate trials. These results indicate that not all LAB groups are able to reduce *Salmonella* within the crop of broiler chickens during a feed withdrawal period.

Experiment 2

LAB candidates were provided as probiotic formulations to 4 week old broiler chickens for 12 hours (during an eight (8) hour feed withdrawal period and the (4) hours preceding the feed withdrawal. All LAB formulations were administered via the drinking water at 10^8 cfu/ml. The chickens were infected with *Campylobacter jejuni* and *Salmonella enteritidis* prior to treatment. Each bird was gavaged with 10^8 cfu *Salmonella enteritidis* the day prior to treatment. Each bird was gavaged with 10^8 cfu *Campylobacter jejuni* 7 days prior to treatment. The crops of the chickens were removed and tested for the presence of *Salmonella enteritidis* and *Campylobacter jejuni*. Briefly, the chickens were humanely euthanized. The crop was aseptically removed and macerated in a sterile bag. Sterile saline was added (4 times the weight of the sample). A sample of the crop/saline solution was taken and serially diluted then directly plated onto brilliant green agar (BGA) for enumeration of *Salmonella* or onto Campy-Cephex agar for enumeration of *Campylobacter*. To the remainder of the crop/saline solution tetrathionate broth was added in an amount to double the initial volume. Both the BGA plates and crops were incubated overnight at 37°C . The Campy-Cephex plates were incubated overnight at 42°C . BGA and Campy-Cephex plates were read and data was recorded. A sample from the crop solution was then struck onto BGA plates and incubated at 37°C overnight for determination of percent of chickens with *Salmonella*. FIG. 9 illustrates the number of colony forming units (cfu) per gram (Log_{10}) of *Salmonella enteritidis* recovered. The crop of the chickens was removed and tested for the presence of *Salmonella enteritidis*. FIG. 10 illustrates the percent of chickens from which *Salmonella enteritidis* was recovered. FIG. 11 illustrates the number of colony forming units (cfu) per gram (Log_{10}) of *Campylobacter* recovered. In this experiment several LAB formulations (34B, 39B, 59B, Combo1, Combo2, and Combo3) were compared to a negative control (Con) that was not treated. In this experiment all treatment groups met the at least 1 log_{10} cfu/g reduction of *Salmonella* in the crop. Similarly, all treatment groups also reduced the incidence as reported as percentage *Salmonella* positive broiler chickens as compared to the negative control. Groups 39B and Combo2 were selected for further study.

Multiple Replicate In Vivo Testing Probiotic Formulation

LAB candidates previously tested in single replicate trials that met the minimum 1 log₁₀ cfu/g reduction of *Salmonella* from crop content were retested for their ability to reduce *Salmonella* and *Campylobacter* in the crop of broiler chickens in a multiple replicate trial. Three replicates pens with 12 chickens each were used for each group. Each bird was gavaged with 10⁸ cfu *Salmonella enteritidis* the day prior to treatment. Each bird was gavaged with 10⁸ cfu *Campylobacter jejuni* 7 days prior to treatment. All LAB formulations were administered via the drinking water at 10⁸ cfu/ml. Briefly, the chickens were humanely euthanized. The crop was aseptically removed and macerated. Sterile saline was added (4 times the weight of the sample). A sample of the crop/saline solution was taken and serially diluted then directly plated onto brilliant green agar (BGA) for enumeration of *Salmonella* or onto Campy-Cephex agar for enumeration of *Campylobacter*. To the remainder of the crop/saline solution tetrathionate broth was added. Both the BGA plates and crops were incubated overnight at 37°C. The Campy-Cephex plates were incubated overnight at 42°C. BGA and Campy-Cephex cfu plates were read and data were recorded. A sample from the crop solution was then struck onto BGA plates and incubated at 37°C overnight for determination of percent of chickens with *Salmonella*. FIG. 12 illustrates the number of colony forming units (cfu) per gram (Log₁₀) of *Salmonella enteritidis* and FIG. 13 the number of *Campylobacter jejuni* recovered. In this trial, LAB candidates were compared to a negative control (Con) which was not treated. LAB candidate 64C (*Pediococcus acidilactici* 64-C1 and *Lactobacillus salivarius* 64-C2) was prepared as a probiotic formulation using a ratio of 90:10 *Pediococcus acidilactici* 64-C1:*Lactobacillus salivarius* 64-C2. Likewise 39B (*Pediococcus pentosaceus* 39B2 and *Lactobacillus salivarius* 39B1) was prepared as a probiotic formulation using a ratio of 90:10 for 39B2:39B1. Combo was a combination of *Lactobacillus salivarius* 59B, *Lactobacillus salivarius* 34B, and 39B (*Pediococcus pentosaceus* 39B2 and *Lactobacillus salivarius* 39B1) in equal proportions. In this study, 64C reduced both *Salmonella* and *Campylobacter* more than the other groups. As such, it was selected for use in a commercial field trial in boiler chickens.

Example 3: Field Trial 1

A total of 16 commercial houses were boot swabbed to identify *Salmonella* positive houses from two eight-house farms. Houses with more than 6/8 positive *Salmonella* swabs were selected for inclusion in the trial. Briefly, the houses were split into quadrants and 2 boot swabs were worn while walking the feed and waterlines of a given quadrant. The boots swabs were then removed and placed in a sterile bag and transported on ice back to the laboratory. Swabs were placed into tetrathionate broth and incubated overnight at 37°C.

After overnight incubation, a sterile loop was used to streak a sample onto XLT-4 agar which was then incubated overnight at 37°C. Plates were evaluated for presence of *Salmonella* and data was recorded. Four houses were selected from each farm, two (2) were the controls and two (2) were treated with the probiotic formulation. Each house had approximately 30,000 birds.

Table 2. Houses Positive for *Salmonella* and Treatment

	Farm 1		Farm 2	
	Number Positive	Treatment	Number Positive	Treatment
House 1	6/8	ND	6/8	Probiotic
House 2	7/8	ND	8/8	Probiotic
House 3	8/8	Probiotic	3/8	ND
House 4	8/8	Probiotic	7/8	Control
House 5	8/8	Control	5/8	ND
House 6	8/8	Control	8/8	Control
House 7	8/8	ND	0/8	ND
House 8	8/8	ND	5/8	ND

ND = not determined

Chickens were treated with either control (no probiotic) or with probiotic formulation at the time the feeders were shut off (~10 hours before live haul). LAB candidate 64C (*Pediococcus acidilactici* 64-C1 and *Lactobacillus salivarius* 64-C2) was prepared as a probiotic formulation using a ratio of 90:10 *Pediococcus acidilactici* 64-C1:*Lactobacillus salivarius* 64-C2. The probiotic formulation was administered through the waterlines during the feed withdrawal period at 10^7 cfu/ml.

At live haul, 100 birds were taken from each flock. The birds were transported via cages for approximately 1.5 hours. The crops were removed and cultured for the presence or absence of *Salmonella*. The crop was aseptically removed and placed into a bag containing tetrastationate broth and incubated at 37°C overnight. A sample from the crop solution was then struck onto XLT-4 plates and incubated at 37°C overnight for determination of percent of chickens with *Salmonella*.

The average of the data demonstrates a significant reduction ($P \leq 0.05$) in the number of *Salmonella* positive birds when treated with the probiotic formulation compared to the control (FIG. 14).

Example 4: In vitro effect on Salmonella recovery and pH after 24 hours**a) Salmonella enumeration**

Enumeration of *S. enteritidis* was performed at 0 hours, 6 hours, and 24 hours. The Salmonella concentration at 0 hours was similar in all groups. After 6 hours of incubation, no differences in Salmonella recovery was detected between the control groups and LAB 39B2 and LAB 59B. All other LAB's groups were statistically different ($P < 0.05$) compared to the control group. LABS's 64C1, 64C2, and the combination of both isolates highly reduced ($P < 0.001$) Salmonella recovery compared to the other groups after 24 hours of incubation. Also, LAB's 39B1 and 39B2 significantly reduced Salmonella recovery ($P < 0.05$) compared to the control group.

Table 2. Effect of different LAB's isolates on Salmonella recovery in an *In Vitro* crop assay model.

Treatment	S. enteritidis recovery (Log10)		
	Mean $\pm$ STDERR		
	0 h	6 h	24 h
Control	2.92 $\pm$ 0.05 a	6.30 $\pm$ 0.18 a	9.23 $\pm$ 0.09 a
LAB 34B	2.96 $\pm$ 0.09 a	5.68 $\pm$ 0.16 bcd	8.75 $\pm$ 0.07 ab
LAB 39B1	2.79 $\pm$ 0.11 a	5.69 $\pm$ 0.13 bcd	8.51 $\pm$ 0.08 b
LAB 39B2	2.79 $\pm$ 0.10 a	6.11 $\pm$ 0.10 ab	8.33 $\pm$ 0.14 b
LAB 59B	2.89 $\pm$ 0.04 a	5.99 $\pm$ 0.07 abc	8.86 $\pm$ 0.08 ab
LAB 64C1	2.96 $\pm$ 0.06 a	5.36 $\pm$ 0.09 d	6.26 $\pm$ 0.24 c
LAB 64C2	2.85 $\pm$ 0.08 a	5.48 $\pm$ 0.15 cd	6.31 $\pm$ 0.12 c
LAB 64C1 and LAB 64C2 (50%:50%)	3.06 $\pm$ 0.05 a	5.37 $\pm$ 0.11 d	6.37 $\pm$ 0.19 c

n= 6 replicates per group

b) pH evaluation

After 24 hours of incubation, pH was recorded with a pH meter (Meter Toledo FiveEasy FE20). All LAB's isolates significantly reduced ($p < 0.05$) the pH of the media compared to the control groups. LAB's 64C1, 64C2, and the combination of both produced a more acidic ($P < 0.05$) environment than LAB's 34B, 39B1, 39B2 and 59B.

Table 3. pH evaluation of media with different LAB's isolates after 24 hours of incubation at 37°C.

Treatment	pH after 24h incubation
	Mean $\pm$ STERR
Control	5.62 $\pm$ 0.03 a
LAB 34B	5.41 $\pm$ 0.02 b
LAB 39B1	5.37 $\pm$ 0.05 b
LAB 39B2	5.40 $\pm$ 0.06 b
LAB 59B	5.37 $\pm$ 0.10 b
LAB 64C1	3.62 $\pm$ 0.01 c
LAB 64C2	3.64 $\pm$ 0.02 c
LAB 64C1 and LAB 64C2 (50%:50%)	3.63 $\pm$ 0.02 c

n= 6 replicates per group

- 5 In conclusion, isolates 64C1, 64C2, and the combination of both isolates were very effective to reduced *Salmonella* recovery after 6h and 24h of incubation at 37°C compared to the control group. These isolates also reduced significantly the pH of the media that may explain their effect on Salmonella recovery. LAB 58B was the only isolate evaluated that did not show any statistical difference at any time point evaluation in this trial although it
- 10 significantly reduced the pH of media after 24 hours of incubation.

CLAIMS

1. A method for reducing the level of *Salmonella* and/or *Campylobacter* in poultry meat comprising the step of administering at least one bacterial isolate to the poultry less than 24 hours prior to slaughter preferably at the time of feed withdrawal or during the feed withdrawal period, wherein the at least one bacterial isolate is selected to reduce *Salmonella* and/or *Campylobacter* in the crop of poultry.
2. The method of claim 1, wherein the at least one bacterial isolate is from the genus *Pediococcus*, or the genus *Lactobacillus*, or a combination thereof.
3. The method of claim 1 or 2, wherein the at least one bacterial isolate is selected from the group consisting of:
- (a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;
 - (b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;
 - (c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;
 - (d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;
 - (e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and
 - (f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof;
- or any combination thereof.
4. The method of any of claims 1-3, wherein the *Salmonella* is *Salmonella Enteritidis*, *Salmonella Typhimurium*, *Salmonella heidelberg*, *Salmonella kentucky*, or any combination thereof.

5. The method of any of claims 1-4, wherein the *Campylobacter* is *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, or any combination thereof.

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6. The method of any of claims 1-5, wherein the administration is via the drinking water.

7. The method of any of claims 1-6, wherein the administration is performed from 5 to 15 hours prior to slaughter, such as from 6 to 14 hours prior to slaughter, such as from 6 to 10 hours prior to slaughter.

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8. The method of any of claims 1-7, wherein the administration is performed less than 10 hours prior to feed withdrawal, such as less than 9 hours prior to feed withdrawal, such as less than 8 hours prior to feed withdrawal, such as less than 7 hours prior to feed withdrawal, such as less than 6 hours prior to feed withdrawal, such as less than 5 hours prior to feed withdrawal, such as less than 4 hours prior to feed withdrawal, such as less than 3 hours prior to feed withdrawal, such as less than 2 hours prior to feed withdrawal, such as less than 1 hour prior to feed withdrawal, such as less than 30 minutes prior to feed withdrawal, or such as at the time of feed withdrawal.

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20

9. A bacterial isolate for reducing the level of *Salmonella* and/or *Campylobacter* in the crop of poultry.

10. The bacterial isolate of claim 9, wherein the bacterial isolate is from the genus *Pediococcus* or the genus *Lactobacillus*, or a combination thereof.

25

11. The bacterial isolate of claim 9 or 10, wherein the bacterial isolate is selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number

30

NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number

NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

35

(c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;

5 (e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius*
10 34B having the deposit accession number NRRL B-67180 or a mutant thereof;
or any combination thereof.

12. A composition comprising a bacterial isolate of any of claims 9-11 and an agriculturally acceptable excipient.

15 13. The composition of claim 12 comprising at least two bacterial isolates (such as at least three, such as at least four, such as at least five, such as at least six) selected from the group consisting of

(a) a *Pediococcus acidilactici* 64-C1 strain having the deposit accession number
20 NRRL B-50962; a strain having all of the identifying characteristics of *Pediococcus acidilactici* 64-C1 having the deposit accession number NRRL B-50962 or a mutant thereof;

(b) a *Lactobacillus salivarius* 64-C2 strain having the deposit accession number NRRL B-67320; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 64-C2 having the deposit accession number NRRL B-67320 or a mutant thereof;

25 (c) a *Lactobacillus salivarius* 39B1 strain having the deposit accession number NRRL B-67178; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 39B1 having the deposit accession number NRRL B-67178 or a mutant thereof;

(d) a *Pediococcus pentosaceus* 39B2 strain having the deposit accession number NRRL B-67179; a strain having all of the identifying characteristics of *Pediococcus pentosaceus* 39B2 having the deposit accession number NRRL B-67179 or a mutant thereof;
30 thereof;

(e) a *Lactobacillus salivarius* 59B strain having the deposit accession number NRRL B-67177; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 59B having the deposit accession number NRRL B-67177 or a mutant thereof; and

(f) a *Lactobacillus salivarius* 34B strain having the deposit accession number NRRL B-67180; a strain having all of the identifying characteristics of *Lactobacillus salivarius* 34B having the deposit accession number NRRL B-67180 or a mutant thereof; or any combination thereof.

5

14. A method for selecting at least one bacterial isolate for reducing *Salmonella* and/or *Campylobacter* levels in poultry comprising the steps of:

(a) identifying a bacterial isolate capable of reducing *Salmonella* and/or *Campylobacter* levels *in vitro*;

10

(b) testing said bacterial isolate *in vivo*; and

(c) selecting a bacterial isolate capable of reducing *Salmonella* and/or *Campylobacter* levels *in vivo*.

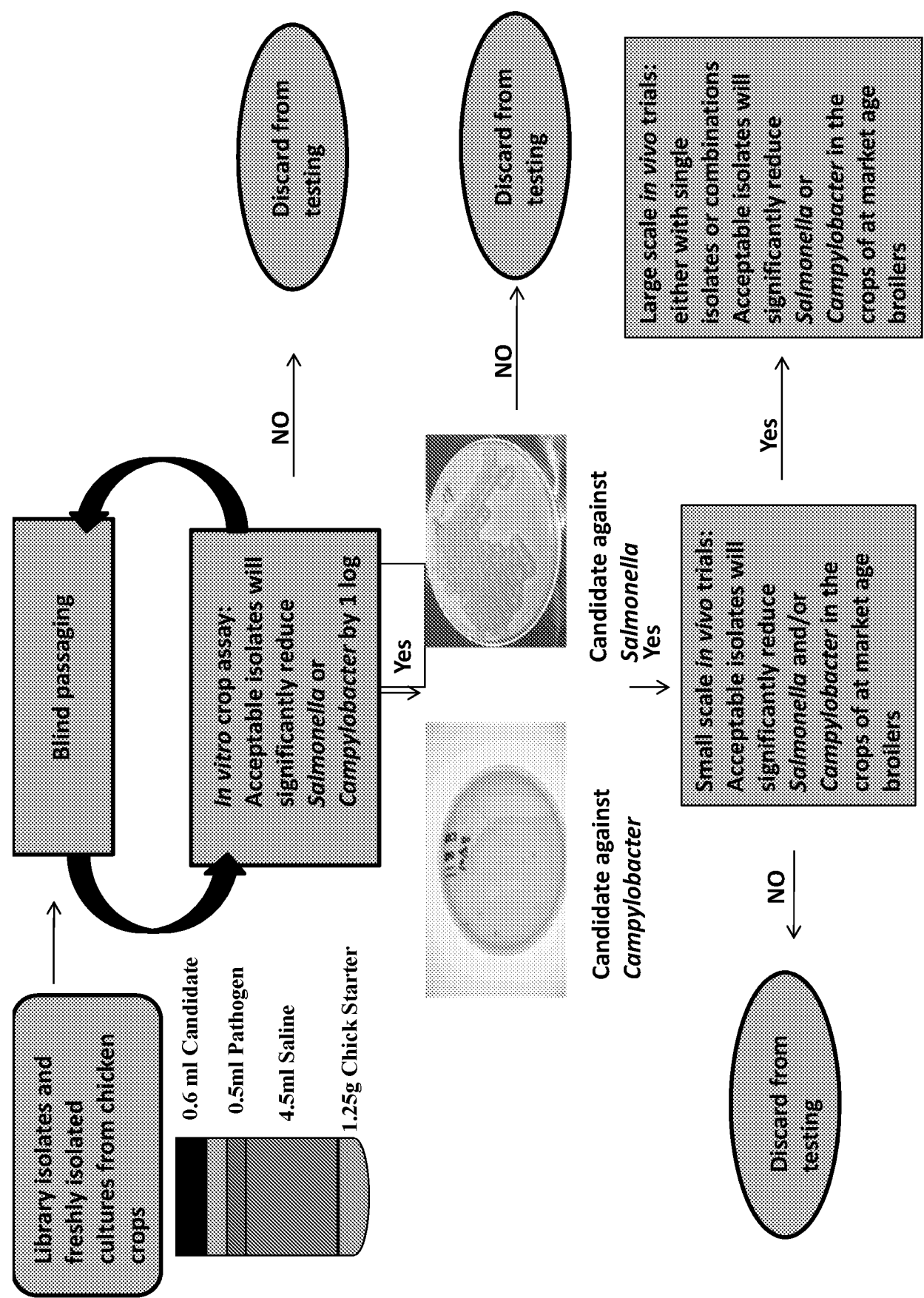
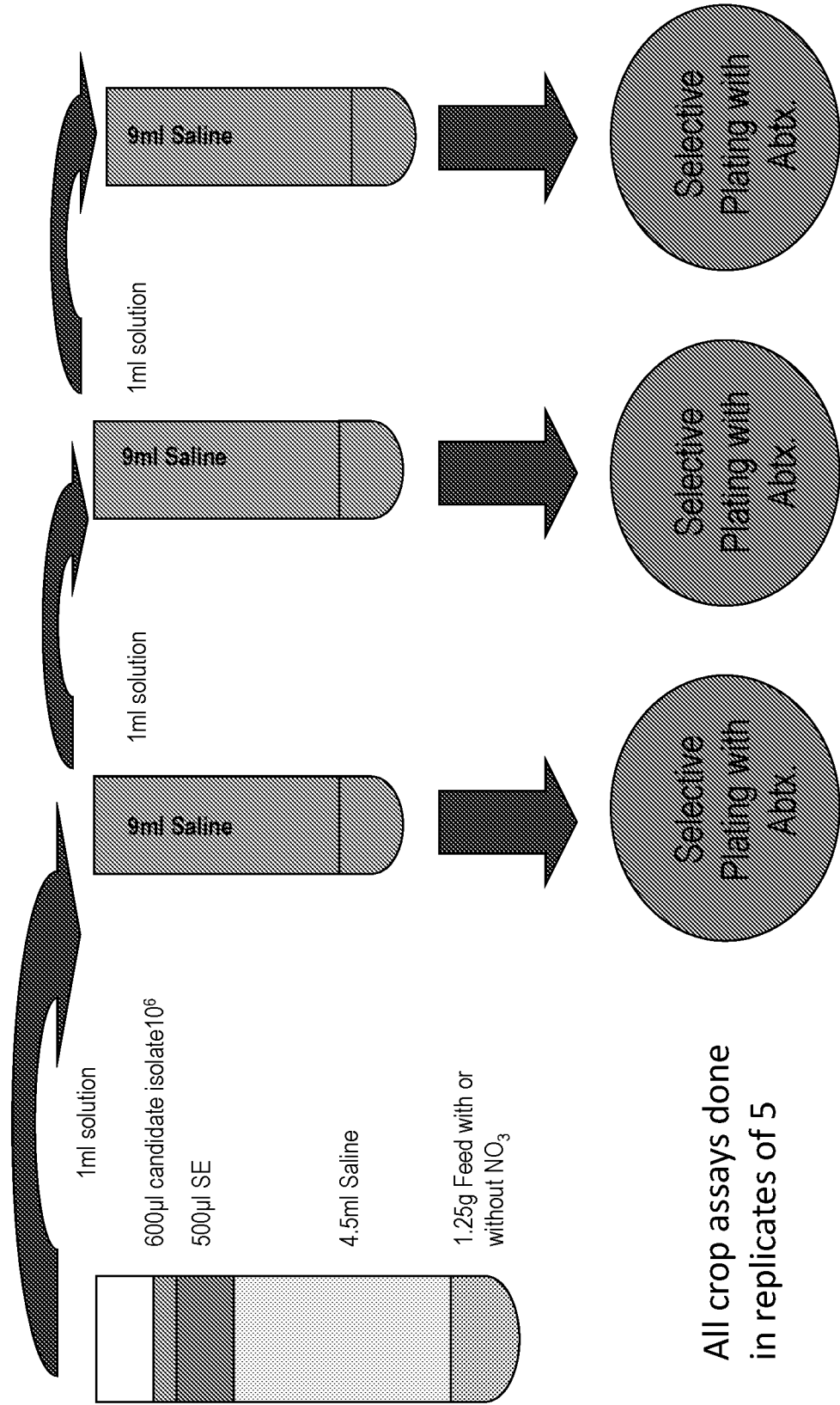


Figure 1

In vitro Crop Assay



All crop assays done in replicates of 5

Figure 2

Effects of probiotic candidates on *Salmonella* Enteritidis in an *in vitro* crop assay at six hours

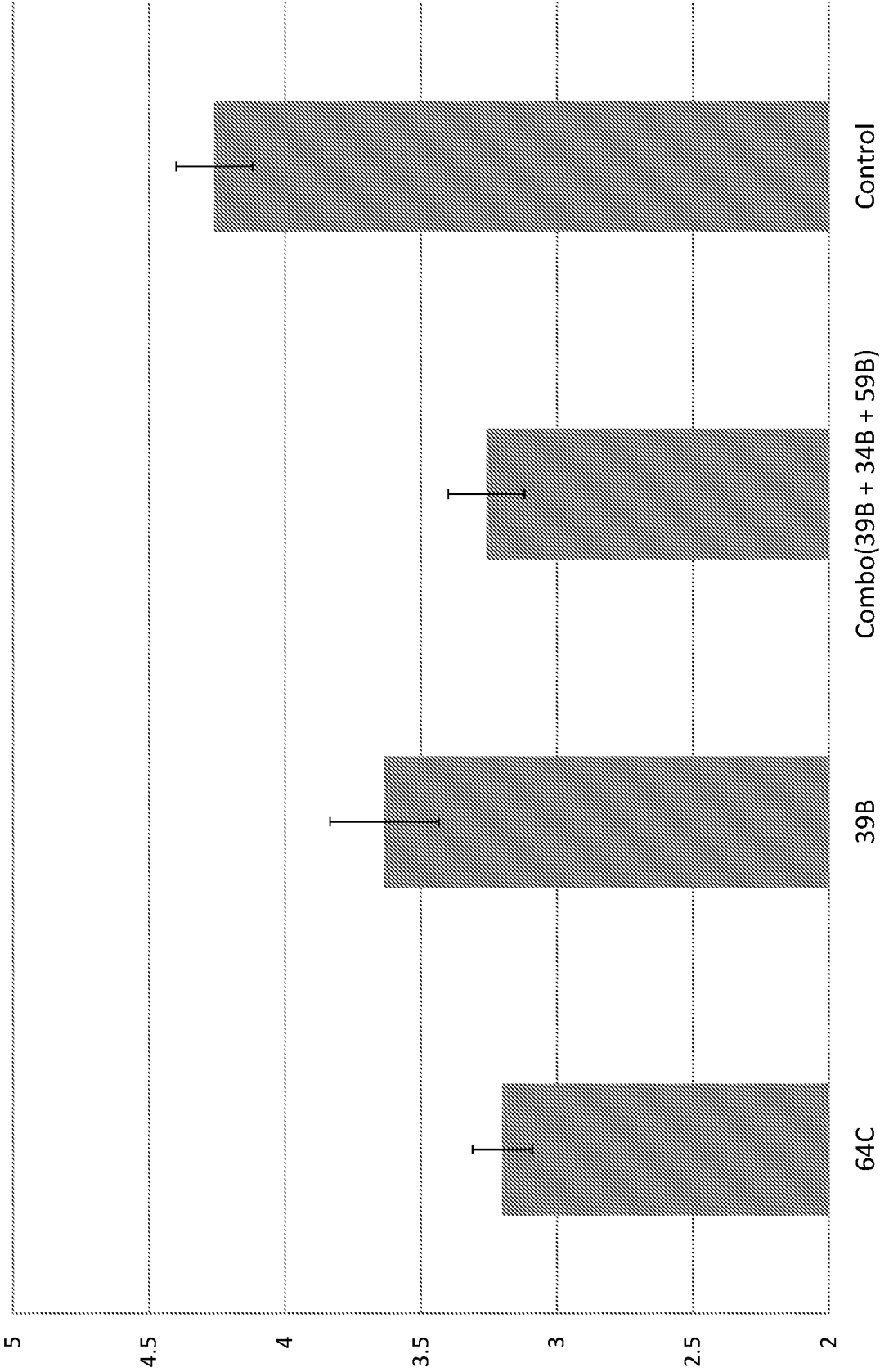


Figure 3

Effects of probiotic candidates on *Campylobacter jejuni* in an *in vitro* crop assay at 42C in buffered saline solution (pH of 6.2) for six hours

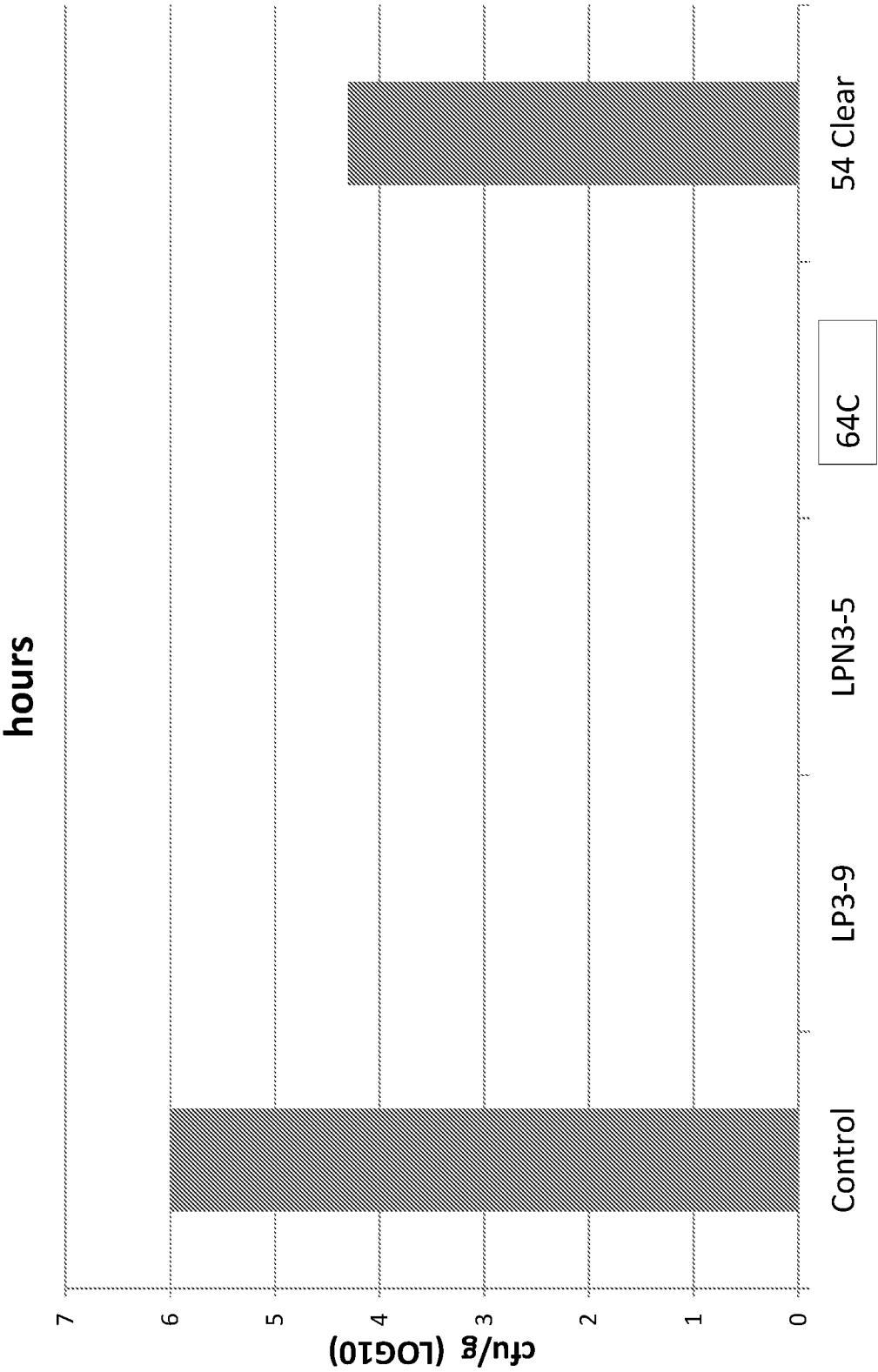


Figure 4

Effects of probiotic candidates on *Campylobacter jejuni* in an *in vitro* crop assay at 42C in saline (pH of 7.2) for six hours

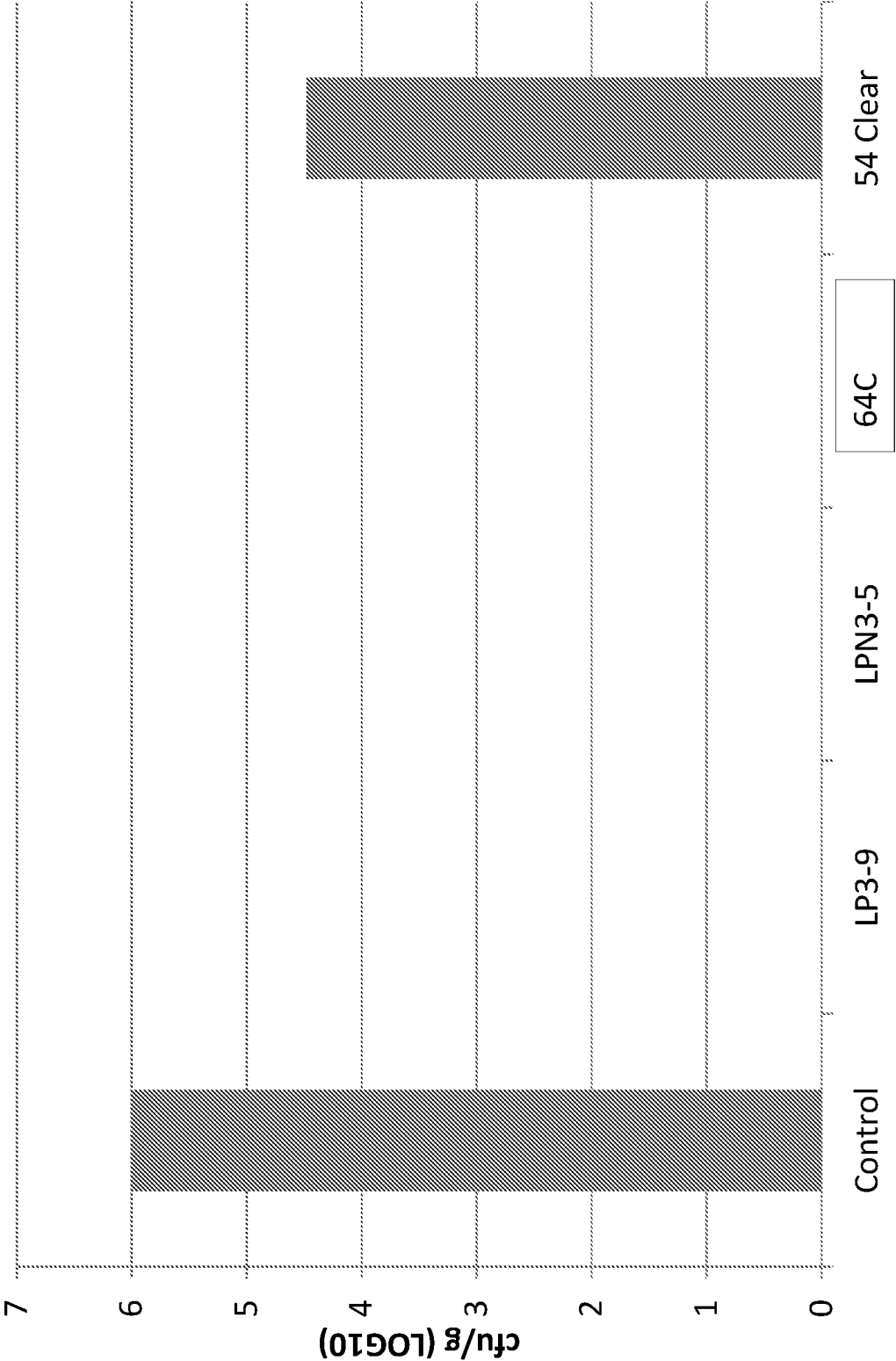


Figure 5

Zones of inhibition by probiotic candidate to common poultry pathogens

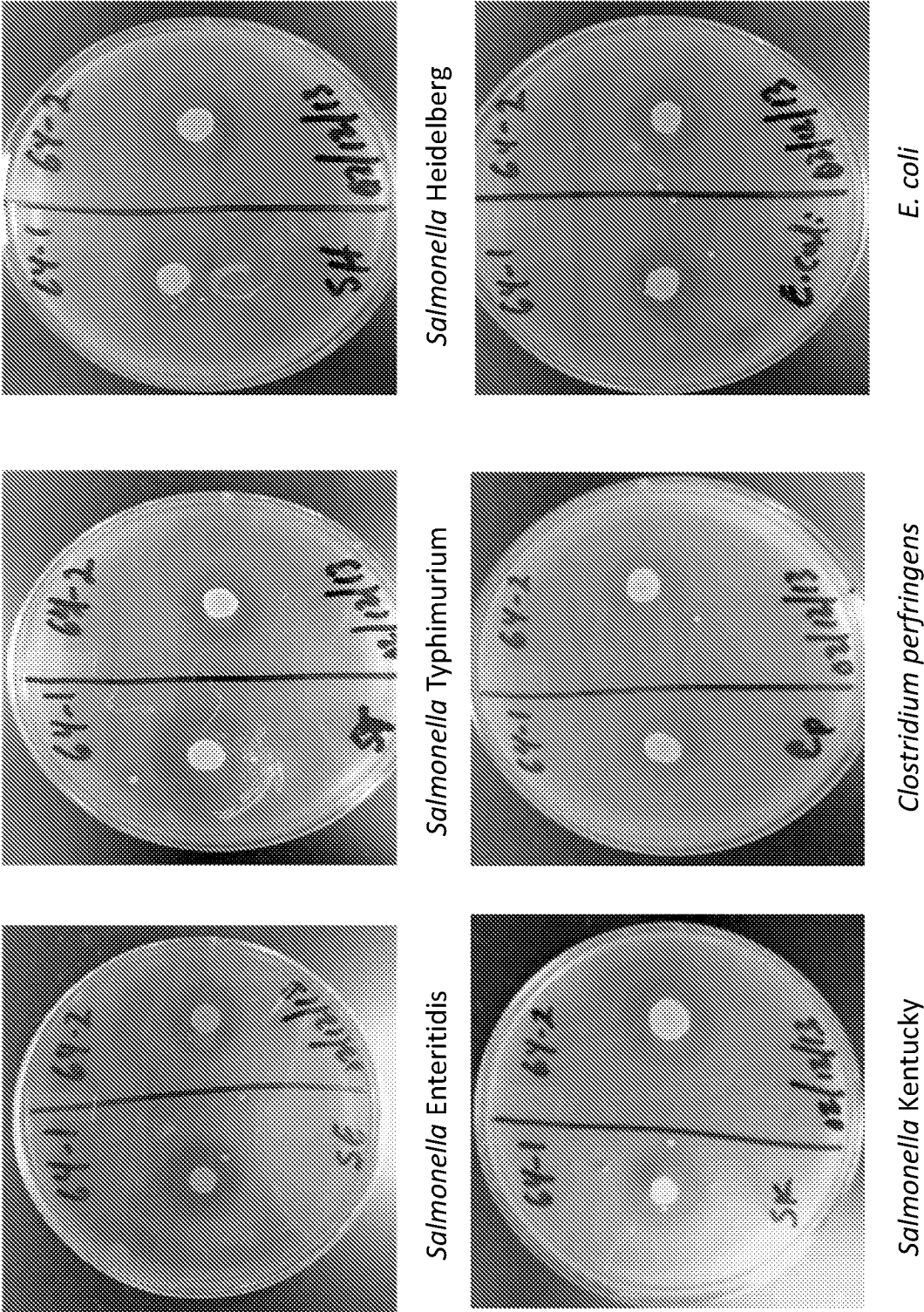


Figure 6

Effect of probiotic candidate formulations on *Salmonella* Enteritidis recovery from crop of 8 week old broiler chickens after an 8 hr feed withdrawal period.

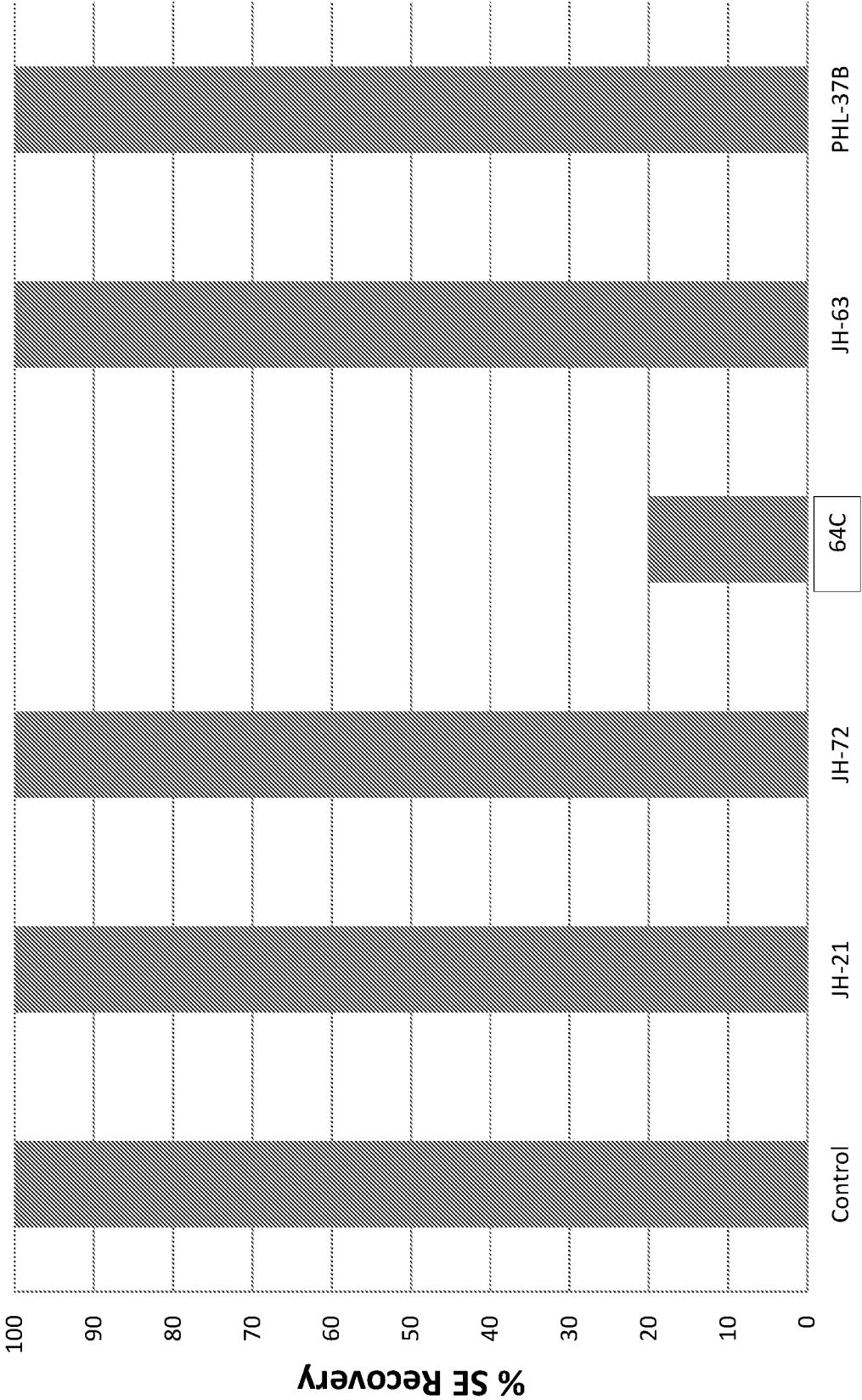


Figure 7

Effect of probiotic candidate formulations on *Salmonella* Enteritidis recovery from crop of 8 week old broiler chickens after an 8 hr feed withdrawal period.

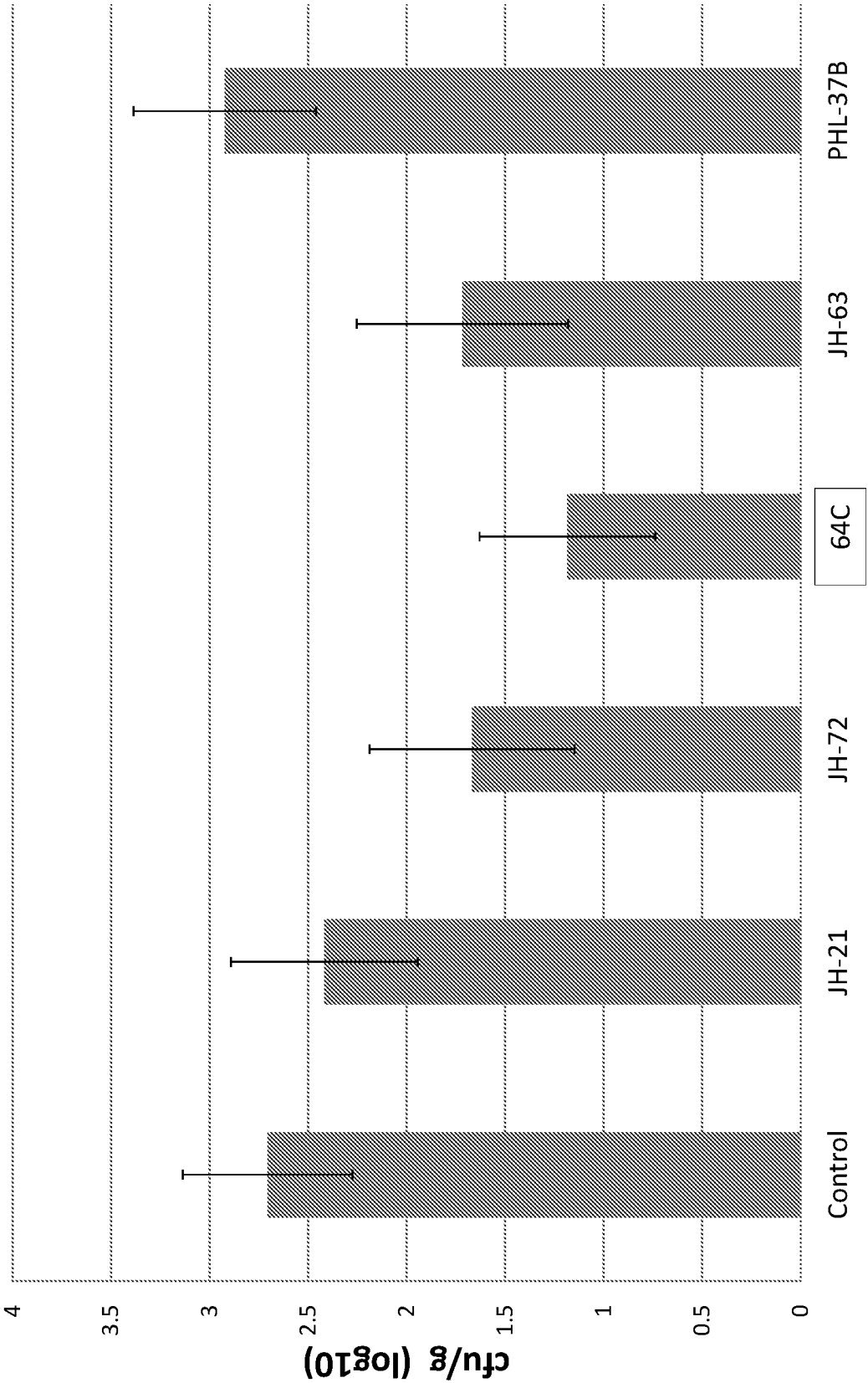


Figure 8

Effect of probiotic candidate formulations on *Salmonella* Enteritidis recovery from crop of 4 week old broiler chickens after an 12 hr feed withdrawal period.

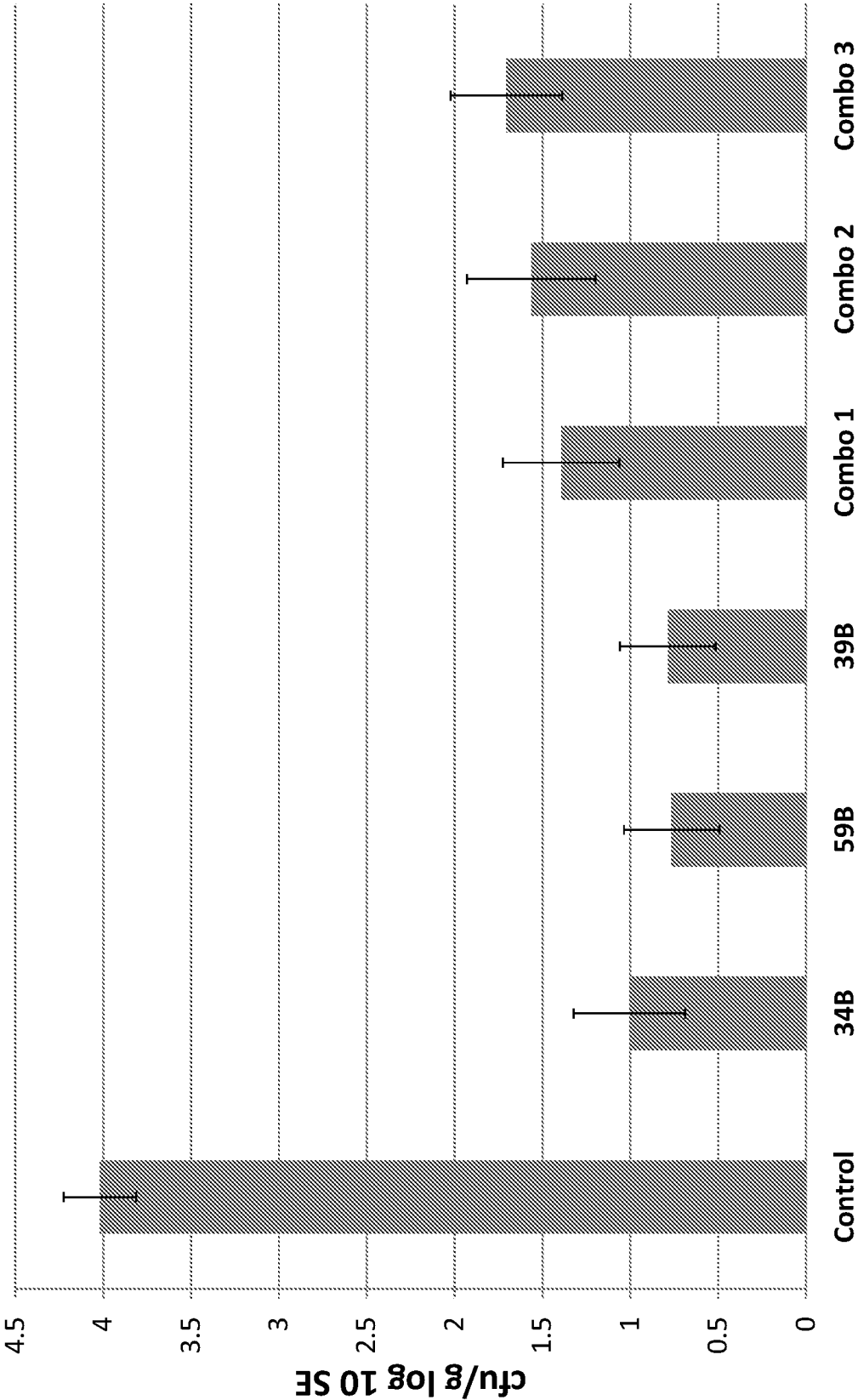


Figure 9

Effect of probiotic candidate formulations on *Salmonella* Enteritidis recovery from crop of 8 week old broiler chickens after an 8 hr feed withdrawal period

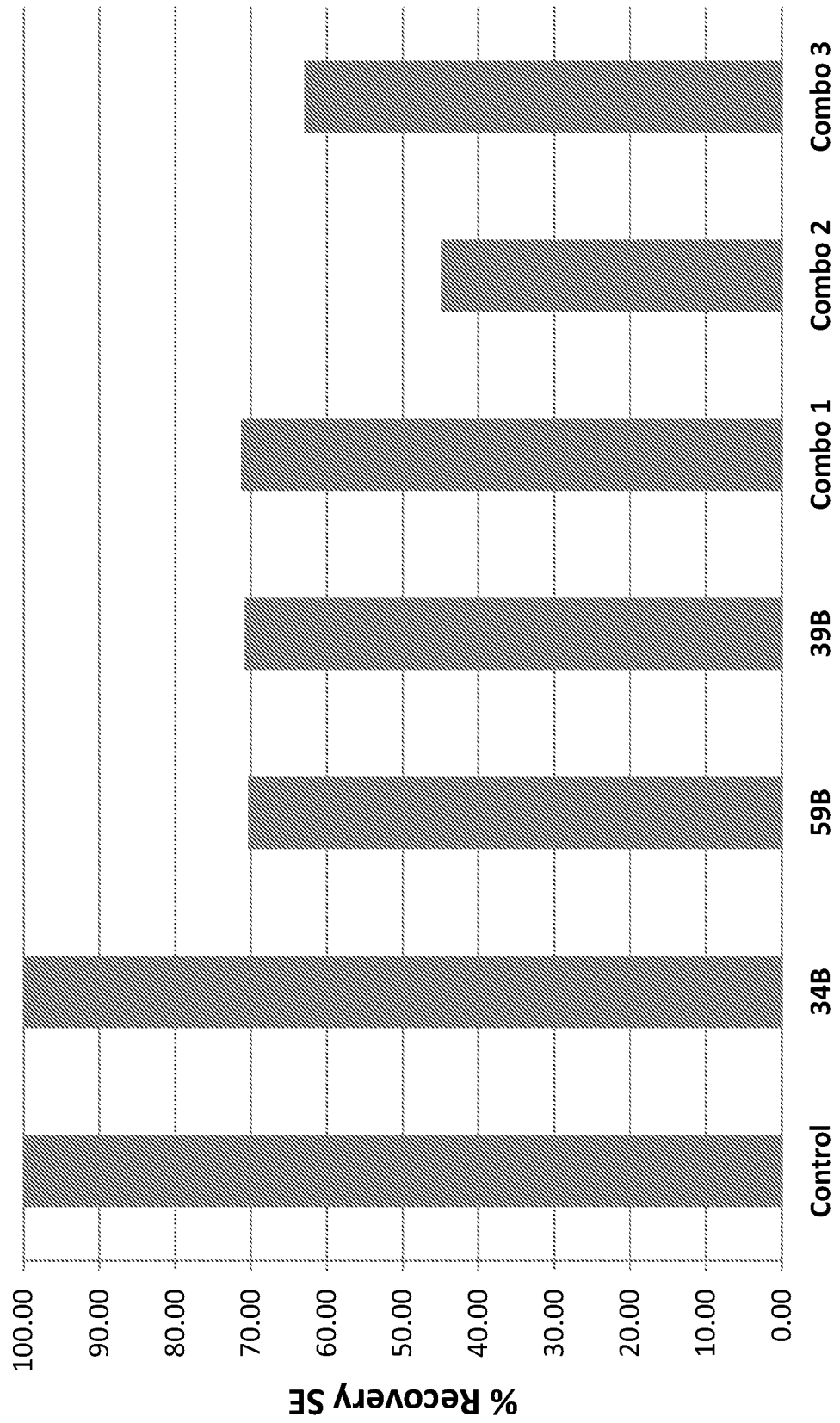


Figure 10

Effect of candidate Labs on reducing *Campylobacter jejuni* in the crop of 4 week old broilers during a 12 hour feed withdrawal period.

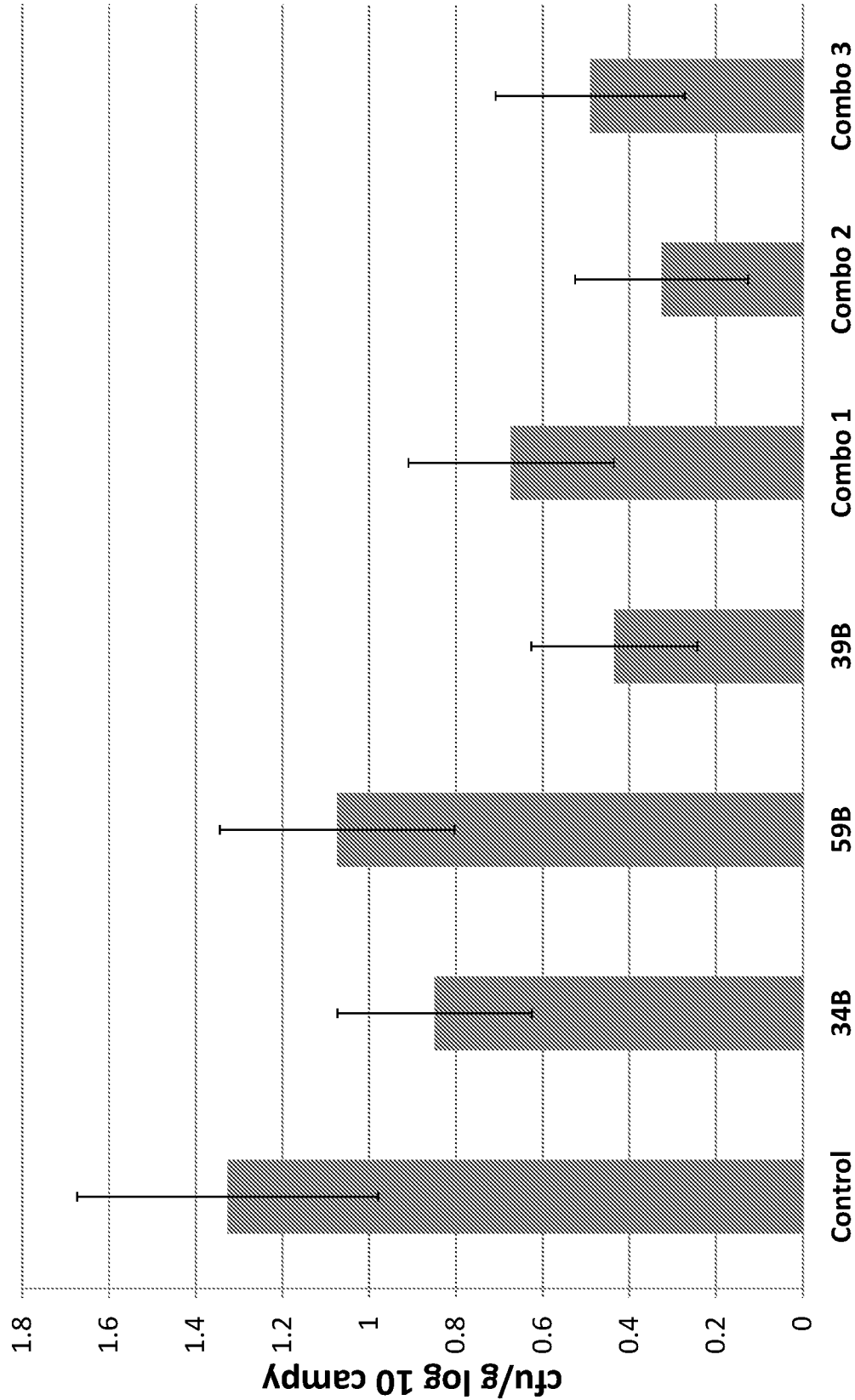


Figure 11

Effect of candidate probiotic on reducing *Salmonella* Enteritidis in the crop of 4 week old broilers during a 12 hour feed withdrawal period

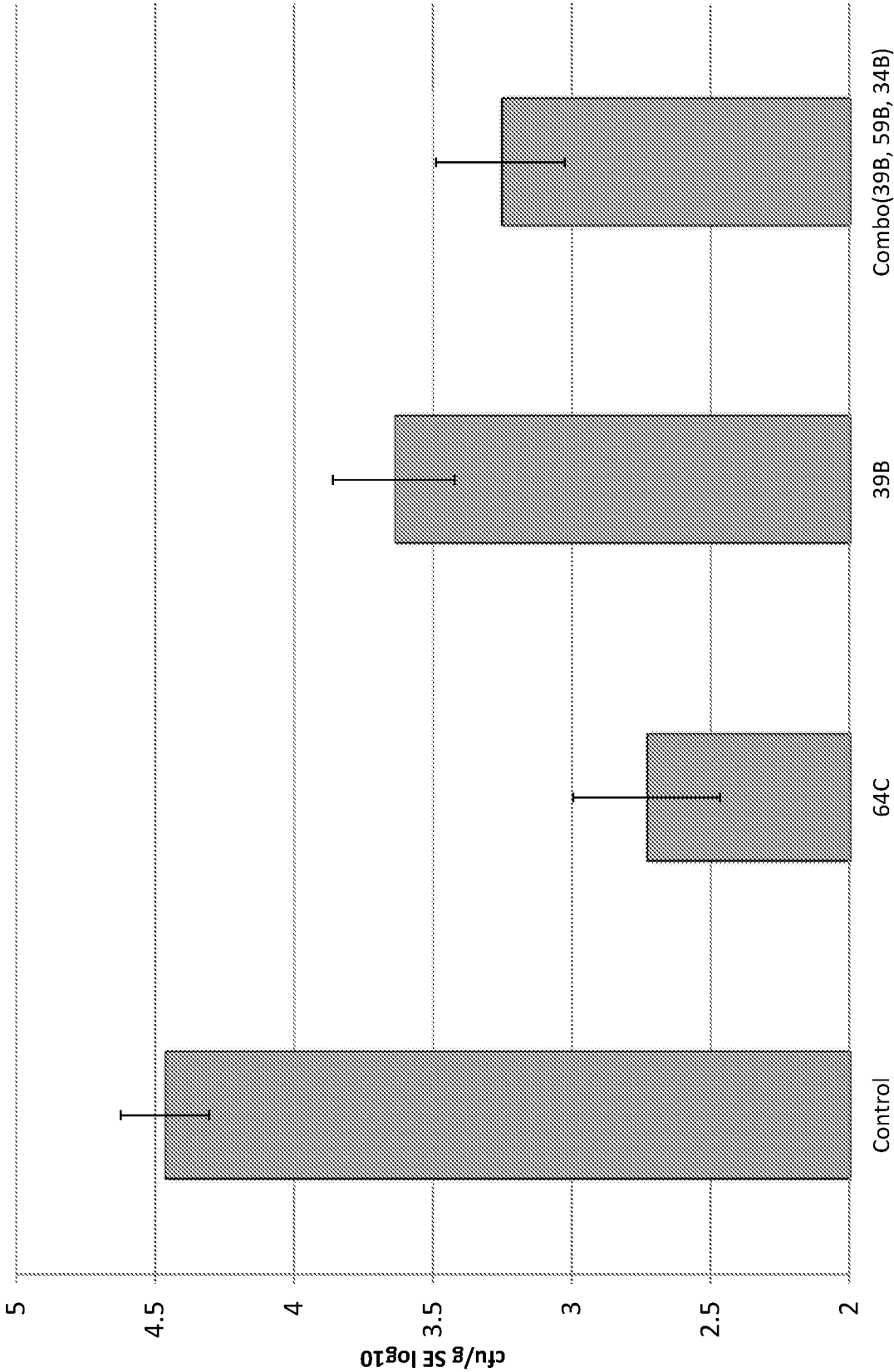


Figure 12

Effect of candidate probiotic on reducing *Camylobacter jejuni* in the crop of 4 week old broilers during a 12 hour feed withdrawal period

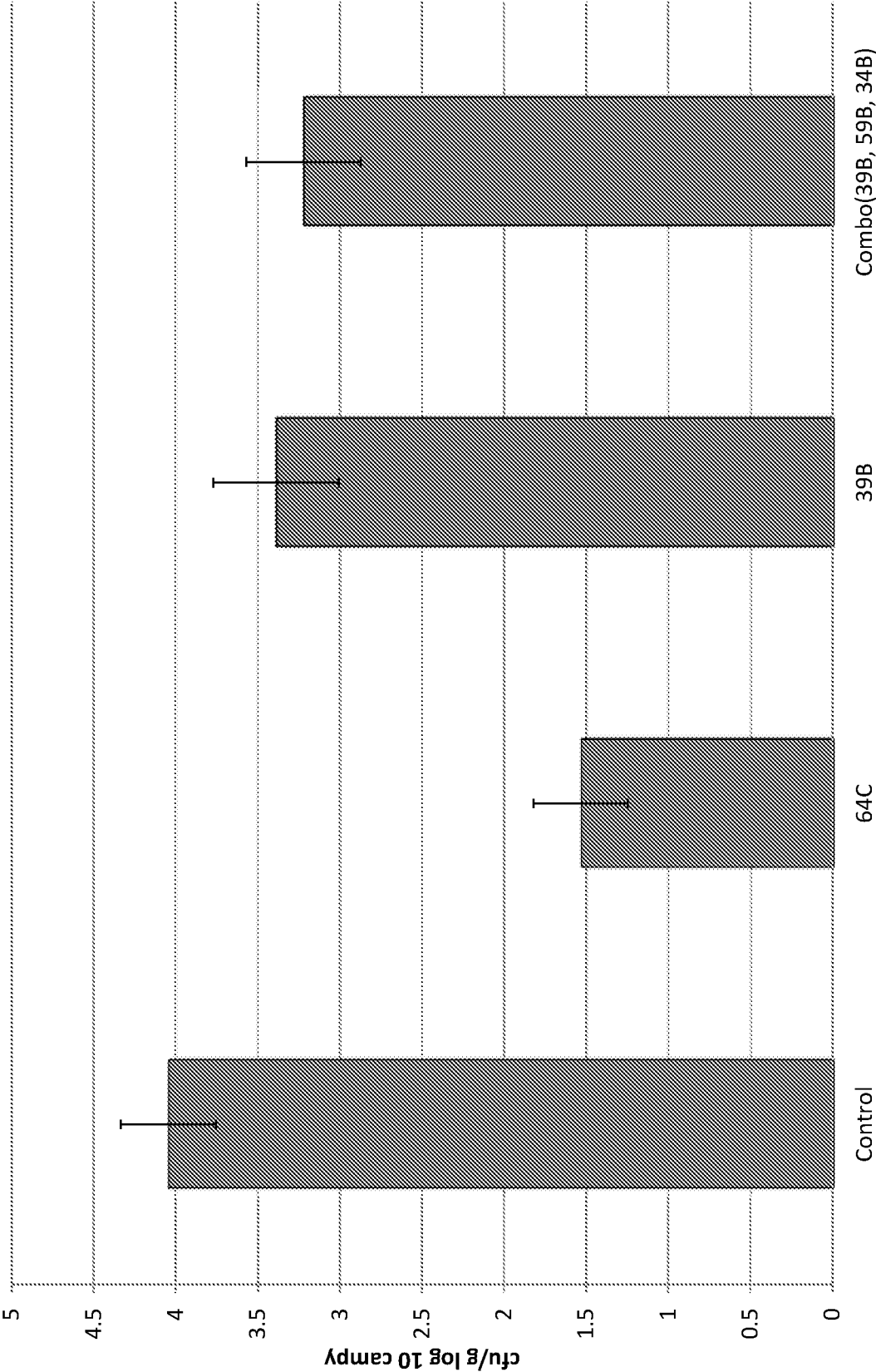
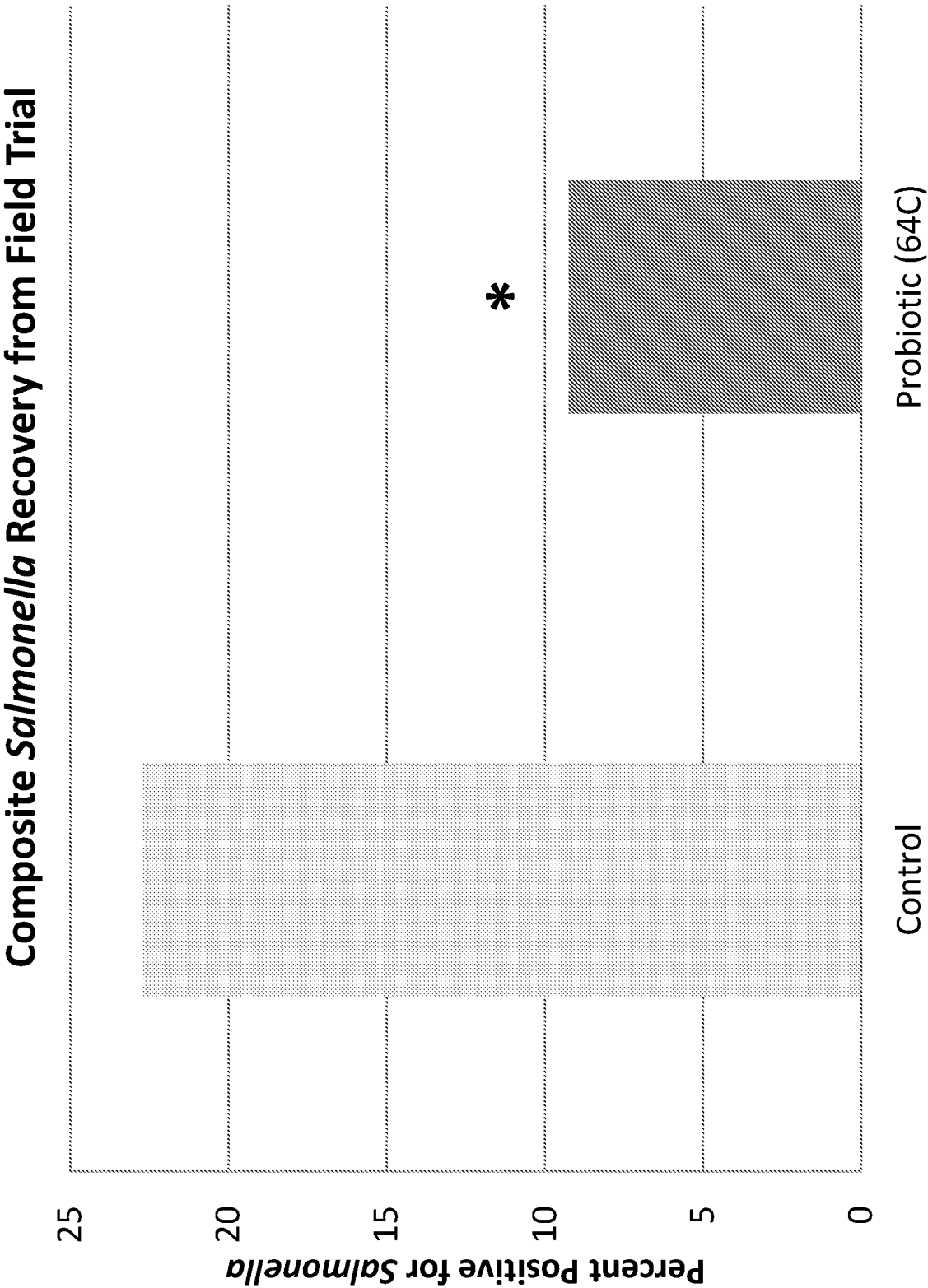


Figure 13



* Denotes significant difference $P \leq 0.05$

Figure 14

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/014838

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K35/744 A23K10/16 A23K50/75 A23K10/18
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/131603 A1 (WARE DOUGLAS R [US] ET AL) 8 July 2004 (2004-07-08)	1,2, 4-10,12, 14
A	Entire document, in particular abstract; para. [0013]; [0019]-[0030]; Ex. 10-11; claims 1-41 ----- -/--	3,11,13



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 March 2017

Date of mailing of the international search report

30/03/2017

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Fax: (+31-70) 340-3016

Authorized officer

Florey, Marianne

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/014838

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E. A. SVETICH ET AL: "Isolation of Lactobacillus salivarius 1077 (NRRL B-50053) and Characterization of Its Bacteriocin, Including the Antimicrobial Activity Spectrum", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 77, no. 8, 15 April 2011 (2011-04-15), pages 2749-2754, XP055011320, ISSN: 0099-2240, DOI: 10.1128/AEM.02481-10	1,2,4-10,12,14
A	Entire document, in particular abstract; page 2750, left col., last two para.; Tab. 4	3,11,13
A	----- S.M. LUTFUL KABIR: "The Role of Probiotics in the Poultry Industry", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 2012 MDPI AG CHE, vol. 10, no. 8, 1 August 2009 (2009-08-01), pages 3531-3546, XP055357284, ISSN: 1661-6596, DOI: 10.3390/ijms10083531 the whole document	1-14
A	----- CONNERTON P L ET AL: "Using antimicrobial cultures, bacteriocins and bacteriophages to reduce carriage of food-borne bacterial pathogens in poultry", 1 January 2011 (2011-01-01), PROTECTIVE CULTURES, ANTIMICROBIAL METABOLITES AND BACTERIOPHAGES FOR FOOD AND BEVERAGE BIOPRESERVATION,, PAGE(S) 181 - 203, XP009190433, the whole document	1-14
A	----- FRANCESCA GAGGIÀ ET AL: "Probiotics and prebiotics in animal feeding for safe food production", INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, vol. 141, 1 July 2010 (2010-07-01), pages S15-S28, XP055126952, ISSN: 0168-1605, DOI: 10.1016/j.ijfoodmicro.2010.02.031 the whole document	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/014838

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004131603 A1	08-07-2004	AU 2003300208 A1	10-08-2004
		US 2004131603 A1	08-07-2004
		US 2008026098 A1	31-01-2008
		US 2011195152 A1	11-08-2011
		US 2013316048 A1	28-11-2013
		WO 2004062388 A1	29-07-2004

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/014838

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments: