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CA 2425236 A1 2004/10/11

(21) 2 425 236

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(22) Date de dépôt/Filing Date: 2003/04/11

(41) Mise à la disp. pub./Open to Public Insp.: 2004/10/11

(51) Cl.Int.^{7}/Int.Cl.^{7} C12Q 1/02, C12Q 1/68, C12Q 1/42, G01N 27/26, G01N 33/48, G01N 33/02

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(54) Titre : METHODE DE DETECTION DE MICRO-ORGANISMES DANS UN BATIMENT ET DE DETERMINATION DE LEURS DEPLACEMENTS ET DE LEUR SOURCE

(54) Title: METHOD OF IDENTIFIYING THE PRESENCE, MOVEMENT AND SOURCE OF MICROBES WITHIN A FACILITY

(57) Abrégé/Abstract:
The present invention relates to a method of identifying the presence, movement and source of microbes within a facility, in particular to a method that uses the genetic fingerprint of microbes to identify their presence, movement and source. The method comprises the steps of: identifying two or more sites in the facility where the microbes may be present; taking swab samples, water samples and/or air samples from designated locations; analyzing bacterial isolates from each of the samples to obtain a genetic fingerprint of the microbes found in each of the samples; and correlating the genetic fingerprints to the sites where the samples were taken and comparing the genetic fingerprints at each location to each other to determine if they are substantially identical.

Abstract:

[0064] The present invention relates to a method of identifying the presence, movement and source of microbes within a facility, in particular to a method that uses the genetic fingerprint of microbes to identify their presence, movement and source. The method comprises the steps of: identifying two or more sites in the facility where the microbes may be present; taking swab samples, water samples and/or air samples from designated locations; analyzing bacterial isolates from each of the samples to obtain a genetic fingerprint of the microbes found in each of the samples; and correlating the genetic fingerprints to the sites where the samples were taken and comparing the genetic fingerprints at each location to each other to determine if they are substantially identical.

METHOD OF IDENTIFYING THE PRESENCE, MOVEMENT AND SOURCE OF MICROBES WITHIN A FACILITY

Field of the Invention:

[0001] The present invention relates to a method of identifying the presence, movement and source of microbes within a facility. In particular, the present invention uses the genetic fingerprint of the microbes to identify their presence, movement and source.

Background of the Invention:

[0002] **a. Pathogens:**

[0003] According to the Center for Disease Control & Prevention, it is estimated that 76 million people develop food poisoning each year in the United States alone (see U.S. Patent No. 6,461,608), and that about 5,000 die as a result. The presence of microbial pathogens in the food supply chain not only affects the health of the local population, but it also represents a potential for spread of these organisms to visitors to the country and to consumers in countries that import food products.

[0004] Prevention of foodborne illnesses by microbial contamination is of major concern to the food industry, regulatory agencies, and consumers. Foodborne microbial contamination occurs both prior to entry into the processing facility, by contamination in the processing environment, and may also occur as a result of events after the food product is made. It is desirable, therefore, to reduce the occurrence and number of foodborne microbes in the food chain. Many means are available for reducing microbial contamination, and antimicrobial treatments are an important component of a food processor's plans to keep microbial growth in check.

[0005] In particular, poultry and meat processors have encountered major difficulties in detecting, preventing and removing microbes that contaminate poultry and meat tissues intended as food products. Of particular concern are *Campylobacter*,

Salmonella, Listeria and pathogenic *E. coli* bacteria. Although *E. coli* bacteria are a major component of normal intestinal flora, certain pathogenic *E. coli* bacteria can cause enteric disease. *E. coli* 0157:H7 bacteria produce a toxin in the intestines that can cause anything from mild diarrhoea to severe hemorrhagic colitis, where the cells of the intestinal lining are damaged, allowing blood to pass into stool. *E. coli* 0157:H7 is found regularly in the feces of healthy cattle, and is transmitted to humans through contaminated food, water, and direct contact with infected people or animals. During the slaughter process, intestinal fluid or feces of infected cattle can drip onto the surface of the meat, contaminating it. If the meat is ground, the harmful bacteria on the surface of the raw meat can become mixed throughout the meat during the grinding process, where it can better survive the heat of cooking. One hamburger patty can contain meat from many cows.

[0006] Certain spoilage organisms, such as *Lactobacillus spp., Pseudomonas spp.* and *Streptococcus spp.* can affect the quality of food products by producing rancidity and off-odours. Food processors typically sample food products or raw materials at specific stages in the food supply chain. In addition, environmental sites are also sampled. If abnormally high levels of contamination are found or a pathogen is detected, remedial steps are taken. A number of intervention strategies are available to treat food at different stages of production and to sanitize the environment to reduce microbial loads. Strategies include irradiation (the treatment of foods by subjecting them to ionizing radiation), pasteurization, chemical treatments (the use of chlorine or chlorine dioxide, ozone, hydrogen peroxide, lactic acid, sodium carbonate, trisodium phosphate, and electrical stimulation) and Ultra Violet light.

[0007] There are many techniques that identify microorganisms, but only two recently developed techniques - pulse-field gel electrophoresis and ribotyping, generate a "genetic fingerprint" of the detected microbe.

[0008] Gel electrophoresis is commonly used in molecular biology to separate molecular components of a sample based on size and charge. Gel electrophoresis is a

standard tool in biochemistry and molecular biology to separate molecular components, such as DNA, RNA or protein, either for subsequent identification or in a preparative procedure. In gel electrophoresis, the different mobility of ions under the influence of an electric field (a function of electrical charge and/or density) serves to separate molecular fragments as they move linearly in the porous medium. It is common to then transfer the separated fragments to a membrane that binds the fragments permitting further processing directed toward making an image or blot visible so that identification can be accomplished. Both pulse-field gel electrophoresis and ribotyping use gel electrophoresis.

[0009] **b. Pulse-Field Gel Electrophoresis**:

[0010] Pulse-Field Gel Electrophoresis, or PFGE, is used to create a DNA "genetic fingerprint" of microbes such as bacteria and fungus. When a sample is taken from either a piece of meat or poultry that is contaminated with a virulent bacterial pathogen, such as *E. coli* O157:H7, L*isteria*, or C*ampylobacter*, it can be cultured to obtain and identify the bacterial isolates. These bacterial isolates are then broken down into their various component parts creating a DNA "fingerprint". The "genetic fingerprint" of the bacteria can then be compared and matched with the "genetic fingerprint" of bacteria found at other locations. When DNA "fingerprints" of the bacteria in the meat or poultry match those at other sites, it provides evidence as to the source of the contamination and/or its movement within a facility.

[0011] **c. Ribotyping:**

[0012] Ribotyping is ribosomal RNA gene fingerprinting. Ribotyping involves the bacterial genes that code for ribosomal RNA. Because such genes are highly conserved, meaning that the genetic information coding for rRNA will vary much less within bacteria of the same strain than it will between bacterial strains, ribotyping has been widely accepted for microbial identification. Ribotyping involves cutting the total genomic bacterial DNA with different DNAases, or restriction enzymes, followed by gel electrophoresis. Following electrophoresis, southern blotting is

performed to blot the DNA bands onto nylon membranes from the gels. DNA probes must be prepared for bacterial 16S and 23S rRNA and labelled with some type of detection system. Membrane hybridization is then performed to hybridize the probes with the appropriate DNA bands on the nylon filter. Difference in the size and location of the ribosomal RNA bands on the filters can then be used to differentiate between organisms and identify sources.

[0013]The RiboPrinter® Microbial Characterization System available from Qualicon, Wilmington Delaware, is a system that uses ribotyping to create an individual genetic "fingerprint" of a microbe. Elements of the RiboPrinter® Microbial Characterization System are described in US Patent No. 4,717,653, US Patent No. 5,306,468, and US Patent No. 5,474,663, all of which are hereby incorporated herein by reference.

[0012] US Patent No. 4,717,653 discloses a method of characterizing an unknown organism by comparing the chromatographic pattern of restriction endonuclease-digester DNA from the unknown organism. The digested DNA has been hybridized or reassociated with ribosomal RNA information-containing nucleic acid from, or derived from, a known probe organism, with at least two equivalent chromatographic patterns, each one of the equivalent chromatographic patterns defining a known different organism species. The species of the unknown organism is established by means of a conserved set of ribosomal RNA sequence-containing restriction fragments present in the chromatographic pattern of the unknown organism.

[0013] Thus, the method of US Patent No. 4,717,653 may be used to characterize and identify species and genera of pathogenic organisms. This method offers an objective way of defining organisms based on detecting DNA fragments containing ribosomal RNA gene sequences in restriction endonuclease digests. The detection is carried out by hybridizing or reassociating DNA fragments with nucleic acid containing rRNA information from a probe organism.

[0014] Briefly, high molecular weight DNA and/or small circular DNAs are isolated from the organism to be identified in order to analyze the rRNA gene sequences and possibly sequences that could be used to create a taxon below the rank of species or infrasubspecific subdivisions. The DNA's are cut at specific sites into fragments, by restriction endonucleases. The fragments are separated according to size by a standard chromatographic system, such as gel electrophoresis. The gels are stained, as is otherwise well known in the art, and standardized as to the fragment sizes using standard curves constructed with fragments of known sizes. The separated fragments are then transferred to cellulose nitrate paper by the Southern blot technique and covalently bound thereto by heating. The fragments containing the rRNA gene sequences are then located, by their capacity to hybridize with a nucleic acid probe containing rRNA information. The nucleic acid probe can either be nonradioactively labelled or (preferably) radioactively labelled. When radioactively labelled, the probe can be ribosomal RNA (rRNA), or radioactively labelled DNA which is complementary to ribosomal RNA (rRNAcDNA), either synthesized by reverse transcription or contained on a cloned fragment, which can be labelled, for example, by nick translation.

[0015] The probe is derived from an arbitrarily chosen organism. Once hybridization has occurred, the hybridized fragments are detected by selectively detecting double stranded nucleic acid (non-radiolabeled probe), or visualized by, e.g. autoradiography (radiolabeled probe). The size of each fragment that has been hybridized is determined from the distance traveled using standard curves. The amount of hybridization, the pattern of hybridization, and the size of the hybridized fragments can be used individually or in conjunction to identify the organism.

[0016] The pattern that emerges from this hybridization can be readily compared to equivalent chromatographic patterns derived from at least two or more genetically well-characterised , organisms, genera or species. After a preliminary broad classification has already been carried out (using, for example, classical taxonomy), the comparison can be either by visual inspection or computer-based and matching of

appropriate chromatographic patterns, by comparison of nucleic acid fragment sizes, by band intensity (amount of hybridization) or by any combination thereof. Ideally, the comparison is carried out with a one-dimensional computer-based pattern recognition system, such as those used in point-of-sale transactions.

[0017] The chromatographic patterns for organisms of the same species are substantially similar, with minor variations allowed for intraspecies differences due to strain variations, whereas differences between species, and differences between genera (and higher classifications) are maximal.

[0018] US Patent No. 5,306,468 discloses an apparatus for the handling and detecting of an electrophoretic transfer membrane that comprises a rigid frame (i) that supports a flexible membrane (ii) along portions of the periphery thereof, the membrane (ii) containing thereon a detectable electrophoretic blot, and a detector therefor. The improvement described in US Patent No. 5,306,468 comprises: (a) guide apparatus designed to accommodate the transfer membrane so that it is positioned for engagement by pressure head (b) and for detection; (b) pressure head positioned with respect to the guide apparatus to releasably engage the membrane (ii); (c) light shielding apparatus positioned with respect to the guide apparatus to selectively enclose about the transfer membrane; and (d) means for sequentially operating the apparatus so that the pressure head engages the membrane (ii) while the transfer membrane is in a position appropriate for detection.

[0019] US Patent No. 5,306,468 also discloses an improved process for the handling and detecting of the electrophoretic transfer membrane. The improved method comprises the steps of: (a) positioning the transfer membrane within guide apparatus for engagement by a pressure head and for detection; (b) engaging the membrane (ii) with a pressure head as the membrane (ii) is resident within said guide apparatus to distort the membrane (ii) from the rigid frame (i); (c) operating light shielding apparatus positioned with respect to the guide apparatus to enclose about the transfer membrane; and (d) detecting the electrophoretic blot, during engagement of the

membrane (ii) with the pressure head as in (b) and operation of the light shielding apparatus as in (c).

[0020] In US Patent No. 5,474,663, an apparatus for direct blot electrophoresis and automated processing and detection is disclosed. The apparatus includes the following components: (a) frame means exhibiting elasticity under compression and having an aperture formed therethrough defining a peripheral surface within the frame means, and further wherein the frame means is bowed; (b) a transfer membrane covering the aperture while in tension and extending beyond all portions of the peripheral surface, the membrane adapted to receive electrophoretically separated molecular fragments; the transfer membrane being secured to the frame means on opposing portions of the peripheral surface sufficient to maintain the frame means in bowed compression and the transfer membrane in tension.

[0021] Also disclosed in US Patent No. 5,474,663 is a process for the preparation of a substantially flat and wrinkle-free membrane for use in automated production of chemiluminescent electrophoretic images. The process includes the steps of: (a) providing a frame means exhibiting elasticity under compression and having an aperture formed therethrough defining a peripheral surface within the frame means, and further wherein the frame means is initially substantially flat; (b) covering the aperture with a transfer membrane of sufficient size to extend beyond all portions of the peripheral surface; (c) bonding the transfer membrane to the frame means along a first portion thereof; (d) bowing the frame means and providing a predetermined tension in the transfer membrane; and (e) bonding the transfer membrane to the frame means along a second portion thereof opposite the first portion and sufficient to maintain the frame means in bowed compression and the transfer membrane in tension.

[0022] **c. Bacterial Source Tracking:**

[0023] Recently, the techniques for obtaining "genetic fingerprints" have been used to try to track the source of bacterial contamination in waterways. A developing

science, referred to as "bacterial source tracking", employs genetic and biochemical tests to allow the likely sources of bacterial contamination of water to be identified. Ribosomal RNA gene fingerprinting (ribotyping) and pulse-field gel electrophoresis are genetic tests that have been used to generate the genetic fingerprints. Examples of bacterial source tracking projects have been carried out by the University of Southern Mississippi and the Virginia Polytechnic Institute and State University.

[0024] Bacterial source tracking has to date been limited to only identifying the source of *E. coli* in waterways.

[0025] There remains a need to provide a method that can identify the presence, movement and source of microbes within any environment, such as a food processing plant.

[0026] The disclosures of all patents/applications referenced herein are incorporated herein by reference.

Summary of the Invention:

[0027] According to one aspect of the invention there is provided a method of identifying the presence, movement and source of microbes within a facility, the method comprising the steps of:

[0028] (a) identifying two or more test sites in the location where the microbes may be present;

[0029] (b) taking swab samples, water samples and/or air samples at the test sites;

[0030] (c) analyzing bacterial isolates from each of the samples to obtain a genetic fingerprint of the microbes found in each of the samples; and

[0031] (d) correlating the genetic fingerprints to the test sites where the samples were taken and comparing the genetic fingerprints at each test site to each other to determine if they are substantially identical.

[0032] In one embodiment of the invention, the step of analyzing the samples is done using ribotyping or pulse-field gel electrophoresis.

[0033] Numerous other objectives, advantages and features of the process will also become apparent to the person skilled in the art upon reading the detailed description of the preferred embodiments and the claims.

Detailed Description of the Preferred Embodiments:

[0034] One objective of one aspect of the present invention is to obtain a baseline evaluation of what microbes are present in a location and where in that location they are found. Some microbes may be directly linked to spoilage, in which case it will usually be economically worthwhile to pursue their sources and eliminate their presence in the location. Some microbes may be pathogens, in which case it is critical to eliminate them from a location such as a food processing plant. Other microbes found may be harmless, but tracking them can help to identify pathways through the location in which microbes travel.

[0035] The preferred method of the present invention provides one or more of the following:

[0036] It provides "genetic fingerprint" identification of microbes, that is, pathogens and spoilage organisms, in a customer's location, such as a plant or food supply chain;

[0037] It allows the source of microbial contamination in the location to be found and eliminated;

[0038] It identifies the pathways of microbial movement within the facility and allows these pathways to be eliminated; and

[0039] It permits users to evaluate their existing sanitation programs to reduce or eliminate microbial contamination to see how effective they are.

[0040] According to a preferred aspect of the present invention, a method of identifying the presence, movement and source of microbes within a location is provided. The location is preferably a food processing plant that processes one or more foods. Most preferably, the foods are selected from the group consisting of meats and poultry such as beef, poultry, pork and lamb; seafood; cereal; confections; cooked, frozen and canned foods; and dairy products, such as cultured, industrial, fluid and ice cream. Alternatively, the location may be a food supply chain preferably selected from the group consisting of: a meat processing supply chain; the processing and packaging of poultry and pork; the growth, processing, packaging and sale of seafood, cereal, confections, and cooked, frozen and canned foods; the provision, preparation and sale of foods at a restaurant chain; and the preparation, delivery, sale and consumption of dairy products, such as cultured, industrial, fluid and ice cream.

[0041] Moreover, the method of the present invention may be useful in other diverse locations, such as hospitals, schools, farms, communities, etc.

[0042] The preferred method of the present invention includes a number of steps as follows:

[0043] (a) Optionally, a map of the location is obtained.. A sampling plan will be prepared to determine where in the location samples should be taken, with two or more test areas identified where microbes may be present. The focus should be on those areas of the location where contamination, or cross-contamination, will most likely result in a safety or spoilage issue. For example, in a food processing plant, this could occur either pre-microbial kill step and post-microbial kill step (ie. Pasteurization or heating product using an oven).

[0044] (b) Taking environmental sample swabs, water samples and/or air samples at the test areas. This is done using standard sterile sampling techniques consistent with the analysis method used.

[0045] (c) Analyzing each of the samples to obtain microbial isolates for genetic fingerprinting. Fingerprinting the microbes is preferably done using ribotyping or pulse-field gel electrophoresis. The microbes may be environmental contaminants, but are preferably pathogens or spoilage organisms that are selected from all microbial species, including various microbes such as *Salmonella*, *Listeria*, including *Listeria monocytogenes, E. coli*, including *E. coli* O157:H7, *Bacillus cereus*, *Lactobacillus,* and *Pseudomonas fluorescens*.

[0046] (d) Correlating the genetic fingerprints to the test areas where the samples were taken and comparing the genetic fingerprints at each test area to each other to determine if they are substantially identical. Microbes that have substantially identical genetic fingerprints originate from the same source.

[0047] (e) Superimposing the genetic fingerprints onto a map of the facility to provide a visual representation of where various microbes are present, and to illustrate how microbes moved within the location.

[0048] (f) By using trace-back analysis, identifying the source of the microbes that have substantially identical genetic fingerprints. This can involve tracking where the microbes are present in the location and where they migrate to by analyzing and comparing the genetic "fingerprints". "Pathways" could be caused by contaminated equipment, such as jigger (similar to a fork-lift truck) wheels, people pathways tracking organisms on their footwear, and plant airflow.

[0049] (g) Identifying what remedial measures can be taken to reduce or eliminate the presence and movement of the microbes in the location, thus improving the quality, safety, shelf life, spoilage, etc. of the product.

[0050] Samples taken at the test areas in the location must be prepared prior to analysis. In the context of the location being a food processing plant, it will be preferable to co-ordinate in advance with the plant laboratory to have samples taken and prepared for analysis. A qualified microbiologist should oversee the preparation to ensure that the samples are prepared properly for subsequent analysis using ribotyping or pulse-field gel electrophoresis.

[0051] Analysis of the samples is preferably done using one of ribotyping or pulse-field gel electrophoresis. Both methods provide a genetic fingerprint of the microbes in the samples.

[0052] **Pulse-Field Gel Electrophoresis:**

[0053] Pulse-Field Gel Electrophoresis, or PFGE, is a method that provides a genetic fingerprint of the microbe. This technique is used to separate the DNA of the bacterial isolate into its component parts. PFGE operates by causing alternating electric fields to run the DNA through a flat gel matrix of agarose. The pattern of bands of the DNA fragments, the "genetic fingerprints", in the gel after exposure to the electrical current is unique for each strain and sub-type of bacteria. By performing this procedure, scientists can identify hundreds of strains of *E. coli* O157:H7 as well as strains of other microbes such as *Listeria* and *Campylobacter*.

[0054] The DNA is first digested into pieces by reacting the isolated DNA with enzymes that are able to specifically break the DNA molecule into individual pieces. The digested DNA is placed at one end of the gel. A pulsing electric field applied across the gel drives the DNA pieces into the gel over a period of hours. The smallest pieces slip through the pores of the gel more quickly, so the pieces are separated as

distinct bands in the gel, based on size. The resulting pattern of 30 to 50 bands, which resembles a bar code is the "fingerprint."

[0055] The DNA "fingerprints" generated by PFGE can distinguish between different strains of microbes. Similar genetic fingerprint patterns suggest that two different bacterial isolates come from a common source, and identifying these connections can help determine the cause of microbial contamination.

[0056] **Ribotyping:**

[0057] As referred to above, ribotyping is ribosomal RNA gene fingerprinting. Ribotyping is a technique based on the analysis of bacterial DNA, which establishes a correspondence between each strain and its genetic fingerprint in the form of a simple bar code; this fingerprint is called ribotype. It involves the bacterial genes that code for ribosomal RNA, and because such genes are highly conserved in microorganisms, ribotyping has been widely accepted for microbial identification. Ribotyping involves cutting the total genomic bacterial DNA with different DNAases, or restriction enzymes, followed by gel electrophoresis. Following electrophoresis, southern blotting is performed to blot the DNA bands onto nylon membranes from the gels. DNA probes must be prepared for bacterial 16S and 23S rRNA and labelled with some type of detection system. Membrane hybridization is then performed to hybridize the probes with the appropriate DNA bands on the nylon filter. Difference in the size and location of the ribosomal RNA bands on the filters can then be used to differentiate between the sources that the fecal bacteria were obtained from.

[0058] Ribotyping has over the past 10-15 years been established as a robust classification and typing method. It was originally developed as a manual method, but took a quantum leap in reproducibility and standardization with the development of the RiboPrinter® Microbial Characterization System by Qualicon (a subsidiary of E.I. DuPont). This instrument automates all of the steps in the process from cell lysis to data capture and database comparisons. Further, reagent cassettes including the enzymes, enzyme conjugated-hybridization probe, electrophoretic gel and membrane

have been developed with the aim of delivering consistent performance. Data capture is accomplished via a CCD camera and the patterns stored in a digitized format. It is, therefore, relatively easy to compare results among laboratories and to exchange data electronically. Using genetic information, the RiboPrinter® system provides an automated genetic snapshot, or RiboPrint® pattern, of any bacterium. It is capable of doing so in less than eight hours.

[0059] RiboPrint® patterns characterize environmental isolates, pathogens, spoilage organisms, control strains, beneficial organisms or any bacterium. Other methods for genetic characterization of bacteria require highly skilled technicians and lack standardization and consistency. The RiboPrinter® Microbial Characterization system provides the speed, accuracy, reproducibility and confidence not achieved with prior systems. The RiboPrinter® system produces an exact genetic snapshot of organisms, allowing the user to identify and track sources of contamination.

[0060] After the samples are prepared, the RiboPrinter® system begins by lysing cells and cutting the released DNA into fragments via a restriction enzyme. These fragments are separated by size through gel electrophoresis and then transferred to a membrane, where they are hybridized with a DNA probe and mixed with a chemiluminescent agent. A digitizing camera captures the light emission as image data, from which the system extracts a RiboPrint® pattern. This pattern is compared to others in the database for characterization and identification, and the results are automatically printed in a report for your review.

[0061] Once the genetic fingerprint of a microbe is obtained, the genetic fingerprint pattern is recorded, digitized and stored in a database. It is variations that exist among microbes in both the position and intensity of rRNA bands that can be used for their classification and identification. Databases for various microbes can be created, such as *Salmonella*, *Listeria*, including *Listeria monocytogenes, E. coli*, including *E. coli* O157:H7, *Bacillus cereus*, *Lactobacillus,* and *Pseudomonas fluorescens*.

[0062] In the context of a food processing plant, for example, contaminating bacteria in finished food products can originate from any one of the raw ingredients, processing personnel or the environment. The method of the present invention allows the establishment of unequivocal relationships between bacterial isolates recovered from any of these sources and the finished product. Moreover, a number of diseases in animals that are caused by bacteria can be traced to the consumption of contaminated feed. The present invention identifies the contaminated feed source for elimination.

[0063] Although the present invention has been shown and described with respect to its preferred embodiments, it will be understood by those skilled in the art that other changes, modifications, additions and omissions may be made without departing from the substance and the scope of the present invention as defined by the attached claims.

What is claimed is:

1. A method of identifying the presence, movement and source of microbes within a facility, the method comprising the steps of:

(a) identifying two or more test sites in the facility where the microbes may be present;

(b) taking swab samples, water samples and/or air samples at the test sites;

(c) analyzing bacterial isolates from each of the samples to obtain a genetic fingerprint of the microbes found in each of the samples; and

(d) correlating the genetic fingerprints to the test sites where the samples were taken and comparing the genetic fingerprints at each test site to each other to determine if they are substantially identical.

2. The method of claim 1, further comprising the step of superimposing the genetic fingerprints onto a map of the facility to illustrate where the microbes are present.

3. The method of claims 1 or 2, wherein microbes that have substantially identical genetic fingerprints originate from the same source, and the method further comprises the step of identifying the source and pathways of movement within the facility of the microbes that have substantially identical genetic fingerprints.

4. The method of any one of claims 1-3, wherein the step of analyzing the samples is done using ribotyping or pulse-field gel electrophoresis.

5. The method of any one of claims 1-4, wherein the facility is a food processing plant, warehouse, distribution centre and the microbes are environmental contaminants, pathogens or spoilage organisms.

6. The method of claim 5, wherein the pathogens or spoilage organisms are selected from all microbial species, including various microbes such as *salmonella*, *Listeria*, including *listeria monocytogenes, E. coli*, including *E. coli* O157:H7, *bacillus cereus*, *lactobacillus,* and *pseudomonas fluorescens*.

7. The method of claims 5 or 6, wherein the food processing plant, warehouse, or distribution centre is a plant/facility that processes or stores one or more foods selected from the group consisting of meats such as beef, poultry, pork and lamb; seafood; cereal; confections; cooked, frozen and canned foods; and dairy products, such as cultured, industrial, fluid and ice cream.

8. The method of any one of claims 1-4, wherein the location is a food supply chain and the microbes are environmental contaminants, pathogens or spoilage organisms.

9. The method of claim 8, wherein the pathogens or spoilage organisms are selected from all microbial species, including various microbes such as *salmonella*, *listeria*, including *listeria monocytogenes, E. coli*, including *E. coli* O157:H7, *bacillus cereus*, *lactobacillus,* and *pseudomonas fluorescens*.

10. The method of claims 8 or 9, wherein the food supply chain is selected from the group consisting of: a meat processing supply chain; the processing and packaging of poultry and pork; the growth, processing, packaging and sale of seafood, cereal, confections, and cooked, frozen and canned foods; the provision, preparation and sale of foods at a restaurant chain; and the preparation, delivery, sale and consumption of dairy products, such as cultured, industrial, fluid and ice cream.