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(54) **Title**: CANINE ORAL CAVITY PROBIOTICS

(57) **Abstract**: The present invention relates to a canine foodstuff or oral care product which comprises a canine oral cavity probiotic, as well as to probiotic strains deposited under the Budapest Treaty, antibodies raised against the strains and which specifically bind to the deposited strains, use of the strains to obtain antibodies, compositions comprising one or more of the probiotic strains and use of the compositions in medicine, a method of detecting the presence of one or more of the strains deposited, kits for detecting the presence of one or more of the strains deposited and a method of improving or maintaining canine oral health. The present invention also relates to other deposited micro-organisms and their use in identifying canine oral cavity probiotics.

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Canine Oral Cavity Probiotics

The present invention relates to a canine foodstuff or oral care product which comprises a canine oral cavity probiotic, as well as to probiotic strains deposited under the Budapest Treaty, antibodies raised against the strains and which specifically bind to the deposited strains, use of the strains to obtain antibodies, compositions comprising one or more of the probiotic strains and use of the compositions in medicine, a method of detecting the presence of one or more of the strains deposited, kits for detecting the presence of one or more of the strains deposited and a method of improving or maintaining canine oral health. The present invention also relates to other deposited micro-organisms and their use in identifying canine oral cavity probiotics.

Oral disease is one of the most common health complications presenting in dogs visiting veterinary clinics in the USA. Periodontal disease is the most widespread oral disease in dogs with incidence increasing with age. The result is that 70-80% of dogs over the age of 3 years demonstrate signs of periodontal disease. The aetiological agent of periodontal disease is dental plaque.

Dental plaque comprises a biofilm of bacteria suspended in a matrix of bacterial exudate and secreted products. Enzymes secreted by plaque bacteria initiate activation of the host immune response, which involves the activation of host matrix metalloproteinases. These host proteases are the major cause of tissue damage and inflammation, which is observed clinically as red and swollen gums or gingivitis. Gingivitis is the initial stage of periodontal disease and without preventative treatment may progress to periodontitis, which is characterised by the destruction of the periodontal ligament and, eventually the supporting tissues including the bone. This phase of the disease is not reversible and tooth mobility and eventual loss will follow in the absence of treatment. The chronic inflammation associated with disease is likely to cause significant pain to the animal in the later stages of periodontitis.

The human oral cavity contains a diverse population of microbes with over 350 different taxa and at least 37 bacterial genera. Human models of dental plaque formation describe primary colonising species binding to the salivary pellicle on the tooth surface. These organisms provide specific attachment sites that can be exploited by secondary colonisers and bridging organisms. In turn, these organisms increase the available binding sites for the tertiary colonisers present in mature plaque, which are often associated with disease. Plaque biofilms therefore comprise a complex ecological mixture of bacterial species that utilise nutrients from the environment and produce metabolites providing a nutrient source for neighbouring organisms.

Despite the relatively detailed knowledge regarding plaque formation and the microorganisms associated with disease in humans, the oral microflora in dogs is relatively undescribed. Several studies have assessed the cultivable species from the canine oral cavity and detected substantial differences in the oral flora of dogs compared to humans. One study described 84 phylotypes from 37 genera cultured from canine plaque and saliva and found only 28% of the organisms identified by 16S rRNA gene sequence were considered indigenous to the human oral flora. In addition, over half of the taxa cultured were novel species with no similar organisms represented in the GenBank database. The majority of organisms cultured were therefore not normally associated with the human oral cavity.

Assessment of the canine microflora has to date been limited to bacterial culture and/or has been targeted through the extrapolation of human studies. Both limitations impact seriously on the scope of research into the canine microflora and the elucidation of organisms associated with disease. This is especially true in light of the apparent differences between the cultivable flora in humans and in dogs, and with approximately 50% of the oral flora representing unculturable species.

The present invention aims to overcome problems in the prior art and to provide novel food compositions and oral care products for canine oral health, as well as methods, kits and agents to identify canine oral health associated micro-organisms.

Accordingly, a first aspect of the invention relates to a canine foodstuff or oral care product which comprises a canine oral cavity probiotic.

A probiotic micro-organism is one which helps to promote a healthy intestinal tract. An oral cavity probiotic is a micro-organism which helps to promote a healthy oral cavity. Probiotic micro-organisms beneficially affect a host by improving the microbial balance. A canine oral cavity probiotic is a micro-organism which achieves this effect in the oral cavity of a canine animal.

The canine foodstuff of the invention is any foodstuff which a canine animal consumes in its diet. The invention covers standard food products as well as food snacks. The foodstuff may comprise a main meal product, a cereal product, a food supplement, drinks or confectionery, such as snack bars, biscuits, chews and sweet products, including candy and chocolate.

The canine animal is preferably a pet dog (*Canis domesticus*).

The foodstuff may be a dry pet food (often referred to as a cereal product). Such dry pet foods include dry kibbles comprising a cooked starch source. The foodstuff of the invention may be a dry product (with approximately 5-12% moisture), a semi-moist product (with approximately 12-70% moisture) or a wet product (with approximately 70-90% moisture). The foodstuff can be provided as a food supplement which is added to another food composition.

The foodstuff may be a cooked product. It may incorporate meat or animal derived materials (such as beef, chicken, turkey, lamb, blood plasma, marrowbone etc., or two or more involved). The composition may alternatively be meat-free (preferably including a meat substitute such as soya, maize gluten or a soya product). The composition may contain additional protein sources, such as soya protein concentrate, milk proteins, gluten etc. The composition may contain a starch source such as one or more grains (for example wheat, corn, rice, oats, barley etc.) or may be starch-free. A typical dry commercial canine foodstuff contains about 30% crude protein, about

10-20% fat and the remainder being carbohydrate, including dietary fibre and ash. A typical wet or moist product contains (on a dry matter basis) about 40% fat, 50% protein and the remainder being fibre and ash. The present invention is particularly relevant for a composition as herein described which is sold as a diet, foodstuff, supplement or treat for a canine animal.

The food supplement may be in the form of a dried powder, tablet, capsule, liquid or gel. The food supplement is preferably added to a foodstuff for the canine animal to consume.

The probiotic micro-organism may be in any form, for example in a powdered dry form or in a spore form (for the micro-organisms which form spores). The probiotic may be encapsulated in order to protect it from moisture or heat. In addition, the probiotic micro-organism may have undergone processing in order for it to increase its survival in any processing step. Accordingly, the micro-organism may be coated or encapsulated in a polysaccharide, fat, starch, protein or in a sugar matrix. The probiotic micro-organism may be in a coating (outer or a layer) or a filling or it may be admixed throughout the composition. It may be preferable to avoid the probiotic being in contact with flour as flour contains enzymes which may adversely affect the viability of the probiotic. Standard encapsulation techniques known in the art can be used, for example as discussed in US 6,190,591 (which is incorporated by reference herein).

The foodstuff of the invention may be a complete and balanced food or may be used in combination with a complete and balanced food (for example, as described in National Research Council, 1985, Nutritional Requirements for Dogs, National Academy Press, Washington DC or Association of American Feed Control Officials, official publication 1996). A complete and balanced diet includes a high quality commercial food. A high quality commercial food can be defined as a diet manufactured to the nutrient requirements of the National Research Council, 1985 (*Supra*), wherein the digestibility of key nutrients is 80% or more.

The foodstuff is preferably packaged. In this way, the consumer is able to identify, from the packaging, the ingredients in the foodstuff. The packaging may be metal (usually in the form of a tin or flexifoil), plastic (usually in the form of a pouch or bottle), paper or card. The amount of moisture in any product may influence the type of packaging which is used or is required.

The oral care product of the invention can be any which is used in the oral care of a canine animal, including canine toothpaste, canine mouth gel or canine mouthwash. Dental care in a canine animal is particularly important. Canine animals have two sets of teeth (similar to humans). The permanent teeth have to last the lifetime of a dog. In the wild state, dogs hunt and chew on fur and bones, but this option is not available for pet canine animals. Ingestion of modern tinned or dry food can leave food deposits in the crevices between teeth and gums, acting as a nutrient for bacteria to thrive in. Plaque and then tartar can develop in these areas and tooth decay will occur. Canine toothpaste, mouthwash and gels are known in the art and any of these can be used to incorporate the oral cavity probiotics of the present invention. Such canine oral care products are distinct from human oral care products. Canine animal owners are advised never to use human products for pet animals as they often contain detergents which can cause foaming and stomach irritation.

Pet toothpaste formulations are available from a number of companies such as CEVA, Petosan and Dentipet. The toothpaste consist essentially of dental grade silica, abrasives, dicalcium phosphate, pumice and one or more thickening and binding gents. In some formulations, the thickening and binding agents comprise one or more agents selected from a group including sorbitol, polyethylene glycol, carboxymethylcellulose, and glycerine. Pet toothpaste formulations exclude surfactants. This is an important innovation, because such surfactants, though harmless to humans, are dangerous for pets such as dogs, if swallowed.
Gels and mouthwashes are also available for pet animals from companies such as Oxyfresh. Mouthwashes are usually composed of Deionized Water (Aqua), Oxygene® (Stabilized Chlorine Dioxide), Zinc Acetate, Sodium Citrate, Chlorophyllin-Copper Complex, Sodium Benzoate, Sodium Hydroxide, Citric Acid.

Mouthgels are usually composed of Water (Aqua), Chondrus Crispus (Carrageenan), Sodium Chlorite (Oxygene® - Stabilized Chlorine Dioxide), Aloe Barbadensis Leaf Juice, Chamomilla Recutita (Matricaria) Extract, Glycerin, Methylparaben, Propylparaben. Toys saturated/stuffed with dental products are also known, examples being Dental Kong and Kong Dental Stick. These products contain grooves and a cavity to load with dental paste/gel to clean teeth and help to reduce plaque.

The composition or oral care product according to the first aspect of the invention may comprise the probiotic micro-organism in any concentration, preferably at a concentration from 10^3 to 10^{15} viable cells per gram of the total composition or product. This concentration of cells provides a suitable concentration for successful colonisation of the oral cavity and providing the optimum health benefit to the animal. An additional probiotic strain may be present at a concentration of from 10^3 to 10^{15} viable cells per gram of the total composition or product.

The canine oral cavity probiotic may be a *Frigovirgula* species or a *Neisseria* species. The *Frigovirgula* species may be *Frigovirgula patagoniensis*. The *Neisseria* species may be *Neisseria canis*. The canine oral cavity probiotics may be one or more of those which have been deposited at the [National Collection of Type Cultures, London, UK] under the Accession No. NCTC 13429 or NCTC 13431.

The present inventors have determined that certain micro-organisms which have been shown to be associated with the healthy canine oral cavity are useful as canine oral cavity probiotics. Accordingly, these probiotic micro-organisms can be added to foodstuffs or oral care products in order to improve the oral health of canine animals.

The present invention also relates, as a second aspect, to the bacterial strains deposited at the National Collection of Type Cultures, London, UK under the Accession No. NCTC 13429 (deposited on 25 July 2008) or NCTC 13431 (deposited on 4 September 2008).

The second aspect also relates to a bacterial strain which has a 16S sequence of 97%

or more identical to the following sequence:

Neisseria sp NCTC 13431 SEQ ID NO:1

AGTCGGACGGCAGCACAGAGAAGCTTGCTTCTTGGGTGGCGAGTGGCGAACGGGTGA
GTAACGCATCGGAACGTACCGAGTAGTGGGGGATAACTGTCCGAAAGGATGGCTAAT
ACCGCATACGCTTTGAGAAGGAAAGCGGGGGATCTTCGGACCTCGCGCTATTCGAGC
GGCCGATGTCTGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCAGT
AGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGCAAGCCTGATCCAGCCATGCC
GCGTGTCTGAAGAAGGCCTTCGGGTTGTAAAGGACTTTTGTCAGGGAAGAAAAGCTT
TGGGTTAATACCCTGGAGTGATGACGGTACCTGAAGAATAAGCACCGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTA
AAGCGAGCGCAGACGGTTTGTTAAGCAGGATGTGAAATCCCCGGGCTCAACCTGGGA
ACTGCGTTCTGAACTGGCAGGCTAGAGTATGTCAGAGGGGGGTAGAATTCCACGTGT
AGCAGTGAAATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGG
ATAATACTGACGTTCATGCTCGAAAGCGTGGGTAGCAAACAGGATTAGATACCCTGG
TAGTCCACGCCCTAAACGATGTCAATTAGCTGTTGGGGTGCATGACACCTTAGTAGC
GTAGCTAACGCGTGAAATTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAA
AGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACG
CGAAGAACCTTACCTGGTCTTGACATGTACGGAATCCTCCAGAGACGGAGGAGTGCC
TTCGGGAGCCGTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGT
TGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCATTAGTTGCCATCATTTAGTTGG
GCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTC
CTCATGGCCCTTATGACCAGGGCTTCACACGTCATACAATGGTCGGTACAGAGGGTA
GCCAAGCCGCGAGGTGGAGCCAATCCCAAAAAACCGATCGTAGTCCGGATTGCACTC
TGCAACTCGAGTGCATGAAGTCGGAATCGCTAGTAATCGCAGGTCAGCATACTGCGG
TGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTGGGGGATA
CCAGAAGTAGGTAGGGTAACCGCAAGGAGCCCGCTTACCACGGTATGCTTCATGACT

The bacterial strain may have a 16S sequence which is 97% or more, 98% or more, 99% or more or 100% identical to the 16S sequence set out in SEQ ID NO:1 (above).

The second aspect of the invention also relates to a bacterial strain having a 16S sequence which is identical to the following sequence:

Frigovirgula sp NCTC 13429 SEQ ID NO:2

TGCTTACCATGCAGTCGAGCGGAGTCATAGTAGAAAGATCTTCGGAAATGGAAATTA
TGTACTTAGCGGCGGACGGGTGCGTAACGCGTGGGTAATCTGCCCTTATCATCGGGA
TAACACAACGAAAGATGTGCTAATACCGAATAACATGCATTTTCGGCATCGAAGATG
TATCAAAGTGTTAGCGGATAAGGATGAGCCTGCGTCTGATTAGCTAGTTGGTGGGGT
AATGGCCTACCAAGGCGACGATCAGTAGCCGACCTGAGAGGGTGATCGGCCACACTG

GAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT
GGGCGCAAGCCTGATGCAGCAACGCCGCGTGAGCGATGAAGGTCTTCGGATCGTAAA
GCTCTGTCGCATGGGAAGATAATGACGGTACCATGTGAGGAAGCCCCGGCTAACTAC
GTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGAATTACTGGGCGT
AAAGGGTGCGTAGGTGGCCTGACAAGTCAGGGGTCAAAGGCAACGGCTCAACCGTTG
TAAGCCTTTGAAACTGTCGGGCTTGAGTTCTGGAGGGGAAATCGGAATTCCTAGTGT
AGCGGTGAAATGCGTAGATATTAGGAGGAACACCGGTGGCGAAGGCGGATTTCTGGA
CAGATACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGG
TAGTCCACGCCGTAAACGATGAGTACTAGGTGTTGGCCCGTTAGGGTCGGTGCCGCA
GTTAACACATTAAGTACTCCGCCTGGGAAGTACGCTCGCAAGAGTGAAACTCAAAGG
AATTGACGGGGACCCGCACAAGTAGCGGAGCATGTGGTTTAATTCGAAGCAACGCGA
AGAACCTTACCTAGGCTTGACATCCCTTTGACCGCAGGGTAATGCCTGCTTTCCCTT
CGGGGACAGAGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCTTTAGTTGCCATCAGGTTATGCTG
GGCACTCTAGAGAGACTGCCGAGGACAACTCGGAGGAAGGTGGGGATGACGTCAAAT
CATCATGCCCCTTATGCTTAGGGCTACACACGTGCTACAATGGCTGTTACAAAGGGT
AGCCAAACCGTGAGGTGGAGCCAATCCCATAAAAACAGTCTAAGTTCGGATTGTAGG
CTGAAACTCGCCTACATGAAGCTGGAGTTACTAGTAATCGCAGATCAGCATGCTGCG
GTGAATGCGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCTGGGGGG
GCCCAAAGTCAACTATCTAACTGCAAAGAGGAAGT

Throughout this text reference is made to 16S sequences, by which is meant 16S rRNA gene sequences. In fact, while reference is made to the sequences being those of 16S rRNA, they are, in fact, DNA coding sequences which are given. Thus, the 16S rRNA gene sequences given contain the nucleotide T, rather than the nucleotide U. Reference in this text to the 16S rRNA (or 16S) sequences should, more correctly, be described as DNA encoding the 16S rRNA sequences or sequences which are the 16S rRNA sequence when the T bases are replaced with U bases. Thus, reference in this text to "a 16S rRNA gene sequence (or 16S sequence) with more than % identity to a sequence reference ..." should read "a 16S rRNA gene sequence (or 16S sequence) with more than % identity to sequence reference ... when the T bases are replaced with U bases".

As an eleventh aspect of the invention (described more fully below), the invention relates to the bacterial strains deposited at the National Collection of Type Cultures, London, UK under the Accession No. NCTC 13428 or NCTC 13430 (deposited on 25 July 2008).

The bacterial strains deposited are novel. Since they are novel, they have not

previously been characterised according to genus and/or species or published as valid species in the Journal of Systemic Microbiology and Evolution. In order to do so for the present application, the taxonomic designation was assigned according to the latest acknowledged bacterial code of the International Union of Microbiological Societies (the IUMS). Classification involved identification of the microbe using 16S rRNA gene sequence including comparison to nearest known organisms, sequence alignment and phylogenetic tree construction for 16S rRNA sequences (Paster and Dewhirst, 1998). The neighbour-joining method (Saitou and Nei, 1987) was then used to construct a phylogenetic tree of the canine-derived micro-organisms. The organisms of the present invention are novel and the method used herein to classify the micro-organisms is constantly changing. In view of this, the micro-organisms could potentially be re-classified in the future. Thus, the name given to the micro-organisms is not limiting, should the micro-organism be re-classified. According to the current classification system, the bacterial strain deposited at the National Collection of Type Cultures, London, UK under the accession number NCTC 13428 is a *Porphyromonas* sp.; the bacterial strain deposited at the National Collection of Type Cultures, London, UK under the accession number NCTC 13429 is a *Frigovirgula* sp. ; the bacterial strain deposited at the National Collection of Type Cultures, London, UK under the accession number NCTC 13430 is a *Peptostreptococcus* sp. and the bacterial strain deposited at the National Collection of Type Cultures, London, UK under the accession number NCTC 13431 is a *Neisseria* sp.

The deposited strains of the present invention have a preferred growth media and conditions:

For the strains deposited at the National Collection of Type Cultures, London, UK under the accession numbers NCTC 13428, NCTC 13429 and NCTC 13430 these are as follows: Colombia agar, supplemented with defibrinated horse blood (5%), menadione (5mg/l) and hemin (5mg/l). The incubation atmosphere is an anaerobic atmosphere of 80% (v/v) nitrogen, 10% (v/v) hydrogen and 10% (v/v) carbon dioxide. The incubation temperature is 37°C with an incubation period of greater than 4 days

being required.

For the strain deposited at the National Collection of Type Cultures, London, UK under the accession number NCTC 13431 and the preferred growth media and conditions are as follows: Columbia agar, supplemented with defibrinated horse blood (5%).The incubation atmosphere is an aerobic atmosphere (typically 78% (v/v) nitrogen, 21%(v/v) oxygen, 0.9% (v/v) argon and 0.04% (v/v) carbon dioxide). The incubation temperature is 37°C with an incubation period of 1 day.

The bacterial strains deposited under the accession numbers NCTC 13429 and 13431 in accordance with the present invention are novel bacteria strains which the inventors have recognised as being associated with periodontal health in canine animals. Since these deposited strains are associated with periodontal health in canine animals, binding partners, specifically antibodies which recognise the deposited strain are particularly useful for detecting the presence of the bacterial strain in the mouth of a canine animal.

Accordingly, a third aspect of the invention relates to an antibody or a part of such an antibody which is raised against any one of bacterial strains or part of these strains according to the second aspect of the invention. The antibodies can be made by any methodology. For example, the antibody can be a polyclonal antibody or a monoclonal antibody produced, for example by the method described in Kohler and Milstein, 1975.

A fourth aspect of the invention relates to an antibody or part of such an antibody which specifically binds any one of the bacterial strains or part of such strains according to the second aspect of an invention. In the present text, the phrase "specifically binds" means that the antibody binds to and recognises the bacterial strain according to the first aspect of the invention or a part thereof and does not specifically recognise any other strain. In practice, the antibody of the third aspect of the invention, whilst specific, may bind to other proteins or parts thereof that share homologous structure . Antibody binding specificity is variable for each antibody in a

pool of polyclonal antibodies or a specific monoclonal antibody. The observed specificity is also dependant on the hybridisation conditions used and visualisation system used. For the purposes of this work an antibody will be seen as specific if it binds only its target protein when used in <1μM concentrations under standard hybridisation conditions. An example of standard hybridisation conditions is hybridisation to protein transferred via Western blot to a positively charged membrane with all washes and hybridisations described below performed at room temperature. The Western Blot is pre-blocked for 1 hour in blocking solution (TBS buffer; 10mM Tris Cl, 150mM NaCl, pH7.5 supplemented with 10% (w/v)non-fat dried milk powder). It is then washed twice for 10 mins each with wash solution (20mM Tris Cl, 500mM NaCl, pH7.5 solution supplemented with 0.05% (w/v) Tween/ 0.2% (w/v) Triton) and a final wash with TBS buffer for 10 minutes. The antibody is then added at <1μm diluted in TBS (typically a 1/1000- 1/2000 dilution of stock antibody) for 1 hour. The blot is then washed as above. Visualisation of the hybridisation are dependant on the commercial system used. In this case an ECL Western Blotting system (Product code RPN2108) utilising a secondary antibody with a horse radish peroxidase conjugate is used according to the manufacturer's protocol (Amersham, UK)

A fifth aspect of the invention provides the use of any one of the bacterial strains or a part thereof according to the second aspect of the invention to obtain an antibody according to the third or fourth aspect of the invention. The bacterial strains or a part thereof can be used in accordance with standard methodology to obtain a polyclonal or monoclonal antibody according to the third and fourth aspects of the invention. The methodology can be any known in the art, including the method of making monoclonal antibodies described by Kohler and Milstein (*supra*).

A sixth aspect of the invention provides a composition comprising one or more of the bacterial strains or a part of such strain according to the second aspect of the invention and/or comprising an antibody, or part of an antibody according to the third or fourth aspects of the invention. The composition is preferably a foodstuff, an oral care product or a pharmaceutical. The composition may comprise the probiotic

micro-organism at a concentration of from 10^3 to 10^{15} viable cells per gram of the total composition. The composition may comprise the antibody in a titre range of from 100 to 5000. The composition may have the preferred features as set out for the first aspect of the invention. According to a seventh aspect of the invention, such a composition can be used in medicine, more particularly in the oral cavity of a canine animal for the purposes of increasing the presence of health-associated bacteria and/or decreasing the presence of disease-associated bacteria and thus improving or maintaining the microbial balance in the canine oral cavity. The composition containing the antibodies, or parts of such antibodies can be used therapeutically, as described in EP-A-0225254 (which is incorporated herein by reference). The antibody or parts thereof can be produced by inoculation of hens, such that the active substance is produced in the egg of the hen. The yolk and/or albumin of the hen can be used to obtain the antibody. Preferably, the yolk and/or albumin is homogenised or agitated to form an emulsion and dried to form a powder which contains the active ingredient (by a conventional technique such as spray drying or lyophilising). The powder is then used in a foodstuff, oral care product or pharmaceutical according to the invention. A pharmaceutical composition according to the sixth aspect of the invention comprises the oral health probiotic and a pharmaceutical excipient. Suitable carriers and/or diluents as excipients are well known in the art and include pharmaceutical grade starch, mannitol, lactose, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose (or other sugar), magnesium carbonate, gelatin, oil, alcohol, detergents, emulsifiers or water (preferably sterile). The composition may be a mixed preparation of a composition or may be a combined preparation for simultaneous, separate or sequential use (including administration).

The compounds according to the invention for use as discussed herein may be administered by any convenient method, preferably orally.

For oral administration, the compounds can be formulated as liquids or solids, for example solutions, syrups, suspensions, emulsions, tablets, capsules, lozenges, dry powder and/or granules.

A liquid formulation will generally consist of a suspension or solution of the compound or physiologically acceptable salt in a suitable aqueous or non-aqueous liquid carrier(s) for example water, ethanol, glycerol, polyethylene glycol or an oil. The formulation may also contain a suspending agent, preservative, flavouring or colouring agent.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and microcrystalline cellulose.

A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, powders, granules or pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule.

Compositions for nasal or oral administration may conveniently be formulated as aerosols, drops, gels and powders. Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomising device. Alternatively the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal once the contents of the container have been exhausted. Where the dosage form comprises an aerosol dispenser, it will contain a pharmaceutically acceptable propellant. The aerosol dosage forms can also take the form of a pump-atomiser.

Conveniently the composition is in unit dose form such as a tablet, capsule or ampoule.

An eighth aspect of the invention provides a method of detecting the presence of a bacterial strain as described according to the second aspect of the invention by use of one or more of bacterial culture or by a detector for one or more of nucleic acid, peptide, carbohydrate, or lipid or by amplification of nucleic acid of the micro-organism or by biochemical or phenotypic (including microscopic examination) profiling. Any one or more of these identifying steps may be preceded by culturing of micro-organism. Such techniques are standard techniques known in the art. Typically, a detector for nucleic acid is a hybridizing nucleic acid. When a nucleic acid detector is utilised, there may be amplification of nucleic acid of the micro-organism before identification with a nucleic acid detector. The nucleic acid detector may be a probe comprising at least 80% or at least 90% or 100% identity with at least 10 sequential residues (or at least 11, 12, 13, 14, 15 or 16 residues) in length from any one of sequences given as SEQ ID NOS:1, 2, 3 or 4.

Detection of nucleic acid sequences can also be carried out by binding partners, such as peptides and antibodies, which are able to recognise and identify the nucleic acid sequences. Alternatively, a detector which recognises and identifies a health or disease-associated micro-organism of the invention can be a peptide, carbohydrate or lipid detector or indeed can be a detector to identify the micro-organism, by any other means. Such methods are again, known in the art, in particular including binding partners, such as antibodies. Preferably, the antibody is an antibody as claimed in the third or fourth aspects of the invention.

The methodology used can be any known in the art, including ELISA based technologies and optical or electrochemical biosensors such as surface plasmon resonance utilising antibodies as sensor molecules (Baac *et al.*, 2006; Lazcka et al., 2007). The method enables the pet owner/carer or vet to identify whether the mouth of a canine animal contains bacteria associated with a healthy oral cavity.

Alternative methodologies for detecting bacterial species-specific molecules are in the public domain. These include carbohydrate binding modules, nucleic-acid and protein

aptamers, molecular imprinted polymers, antimicrobial peptides, cellular protein receptors, whole cells and organisms (as reviewed Ngundi *et al* 2006).

A ninth aspect of the invention provides a kit for detecting the presence of the bacterial strain according to the second aspect of the invention, comprising an agent to determine the presence of the strain. The kit may comprise one or more agents. The detector is preferably a detector for nucleic acid, peptide, carbohydrate or lipid as described above according to methods of the invention. Preferably the agent is an antibody or part of an antibody according to the third or fourth aspects of the invention.

The kit may comprise other components and/or may include instructions for use.

A tenth aspect of the invention provides method of improving or maintaining canine oral health in a canine animal, the method comprising administering to said canine animal a foodstuff according to the first aspect of the invention or applying an oral care product according to the first aspect of the invention to the animal. Alternatively a foodstuff, oral care product or pharmaceutical according to the sixth aspect of the invention can be provided to the animal. The canine animal is likely to be in need of having its oral health improved or maintained.

An eleventh aspect of the invention relates to (as described above) bacterial strains deposited under the accession numbers NCTC 13428 and 13430. These strains have been identified as disease-associated strains which are associated with diseased canine oral cavity. These strains are particularly useful in identifying canine oral cavity probiotics. They could also be used in the manufacture of vaccines for immunising canine animals against canine oral cavity disease. The eleventh aspect of the invention also relates to a bacterial strain having the 16S sequence identical to the following sequence:

Porphyromonas sp NCTC 13428 SEQ ID NO:3

GACTTAGCCCCAGTCACCGGTATTACCCTAACACGCCCCTCGCGGTTACGTGCTTTA
GGTACTCCCGACTCCCATGGCTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATT
CACCGCGCCATGGCTGATGCGCGATTACTAGCGAATCCAGCTTCACATAGTCGAGTT
GCAGACTACGATCCGAACTGGGATGGGGTTTGGAGATTCGCATCTTGTCACCAAGTA
GCTGCCCTTTGTCCCCACCATTGTAACACGTGTGTCGCCCCGGATGTAAGGGCCGTG
CTGATTTGACGTCATCCACACCTTCCTCGCGCCTTACGACGGCAGTCTTGTCAGAGT
CCTCGGCTTTACCCGATAGTAACTGACAACGTGGGTTGCGCTCGTTATGGCACTTAA
GCCGACACCTCACGGCACGAGCTGACGACAACCATGCAGCACCTACTTAGCGGCCTT
GTGACAGGATATGTACTTTCATACATAGTCCGCTAAATTTCAATCCCGGGTAAGGTT
CCTCGCGTATCATCGAATTAAACCACATGTTCCTCCGCTTGTGCGGGCCCCCGTCAA
TTCCTTTGAGTTTCACCGTTGCCGGCGTACTCCCCAGGTGGATAACTTAACGCTTTC
GCTCAGGAGCATACTGTATATCGCATACGCCCAGTTATCATCGTTTACTGCGTGGAC
TACCAGGGTATCTAATCCTGTTTGATACCCACGCTTTCGTGCTTCAGTGTCAGTCAT
AACTTAGTAAGCTGCCTTCGCAATCGGAGTTCCTCGTGATATCTATGCATTTCACCG
CTACACCACAAATTCCGCCTACTTCATCTATACTCAAGCCAAACAGTTTCGAAGGCA
AGACTGTGGTTGAGCCACAGCTTTTCACCCCCGACTTATCAGGCCACCTACGCACCC
TTTAAACCCAATAAATCCGGATAACGCTCGCATCCTCCGTATTACCGCGGCTGCTGG
CACGGAGTTAGCCGATGCTTATTCATAGGGTACTTGCAAATATCCACGCGTGGATAA
CTTTAATCCCCTATAAAAGAAGTTTACAATCCATAGAACCGTCTTCCTTCACGCGAC
GTGGCTGGTTCAGGCTCTCGCCCATTGACCAATATTCCTCACTGCTGCCTCCCGTAG
GAGTCTGGTCCGTGTCTCAGTACCAGTGTGGGGGATAAACCTCTCAGTTCCCCTATC
CATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAACTAATGGAACGCATGCCTAT
CTGATAGCAATTAATCTTTACTTGAATCATCATGCAATAACCCAAGACCATAAGGTA
TTAGTCCGACTTTCACCGGGTTATCCCTTTCTATCAGGCAAGTTACATACGCGTTAC
GCACCCGTGCGCCGGTCGCCATCAGTCTTAGCAAGCTAAGACCATGCTGCCCCTCGA
CTTGCATGTGTTAAGCCTATCGCTAGCGTTCATCCTGAGCCAGAATCAAACTCTCA

or a bacterial strain having the 16S sequence identical to the following sequence:

Peptostreptococcus sp. NCTC 13430 SEQ ID NO:4

TGCAAGTCGAGCGCGGTTGTGCTTAGTATTGAGTATTTTATCCATGATAAAATATTG
AATTCTATGCACAACTGAGCGGCGGACGGGTGAGTAACGCGTGGGTAACCTGCCCTA
TACACATGGATAACATACTGAAAAGTTTACTAATACATGATAATATAGTTTTTCGGC
ATCGAAGAATTATCAAAGTGTTAGCGGTATAGGATGGACCCGCGTCTGATTAGCTAG
TTGGTGAGATAACTGCCCACCAAGGCGACGATCAGTAGCCGACCTGAGAGGGTGATC
GGCCACATTGGAACTGAGACACGGTCCAAACTCCTACGGGAGGCAGCAGTGGGGAAT
ATTGCACAATGGGCGCAAGCCTGATGCAGCAACGCCGCGTGAACGATGAAGGTCTTC
GGATCGTAAAGTTCTGTTGCAGGGGAAGATAATGACGGTACCCTGTGAGGAAGCCCC
GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCTAGCGTTATCCGGATT
TACTGGGCGTAAAGGGTGCGTAGGTGGTCTTTCAAGTCGGTGGTTAAAGGCTACGGC
TCAACCGTAGTAAGCCTCCGAAACTGTTAGACTTGAGTGCAGGAGAGGAAAGTGGAA
TTCCCAGTGTAGCGGTGAAATGCGTAGATATTGGGAGGAACACCGGTAGCGAAGGCG
GCTTTCTGGACTGCAACTGACACTGAGGCACGAAAGCGTGGGTAGCAAACAGGATTA
GATACCCTGGTAGTCCACGCCGTAAACGATGAGTACTAGGTGTCGGGGGTTACCCCC
CTCGGTGCCGCAGCTAACGCATTAAGTACTCCGCCTGGGGAGTACGCACGCAAGTGT
GAAACTCAAAGGAATTGACGGGGACCCGCACAAGTAGCGGAGCATGTGGTTTAATTC

GAAGCAACGCGAAGAACCTTACATAAGCTTGACATCCCTCGGACCGGTGTTTAATCA
CACCTTTCTTTCGGGACTGAGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCTTTAGTTGCCATCA
TTAAGTTGGGCACTCTAGAGAGACTGCCAGGGACAACCTGGAGGAAGGTGGGGATGA
CGTCAAATCATCATGCCCCTTATGCTTAGGGCTACACACGTGCTACAATGGGTGGTA
CAGAGGGTTGCCAAACCGTGAGGTGGAGCCAATCCCTTAAAGCCACTCTCAGTTCGG
ATTGTAGGCTGAAACTCGCCTACATGAAGCTGGAGTTACTAGTAATCGCAGATCAGA
A

A twelfth aspect of the invention relates to the use of a bacterial strain according to the eleventh aspect of the invention in identifying canine oral cavity probiotics. The strains according to the eleventh aspect can be used in an assay as shown here in the example to determine if a microbial strain reduces biomass of the disease-associated strain and thus has canine oral cavity probiotic activity.

All preferred features of the different aspects of the invention apply *mutatis mutandis*.

In accordance with the present invention, a canine oral cavity probiotic can also be described as a micro-organism which beneficially affect a canine animal by reducing the biomass of one or both of the disease-associated bacteria species as referred to in the example according to an assay as described in the example.

The present invention is described with reference to the figures, in which:

Figure 1 is a bar chart of total biomass in biofilms of *Peptostreptococcus* sp. NCTC 13430 in co-culture with either *Frigovirgula* sp. NCTC 13429 or *Neisseria* sp. NCTC 13431. Total biomass as measured by crystal violet staining.

Figure 2 is a bar chart of total biomass in biofilms of *Porphorymonas* sp. NCTC 13428 in co-culture with either *Frigovirgula* sp. NCTC 13429 or *Neisseria* sp. NCTC 13431. Total biomass as measured by crystal violet staining.

The present invention is now described with reference to the following, non-limiting examples:

Example

Experiments were conducted in *in vitro* models representing canine dental plaque.

Assay set-up

Periodontal disease associated bacterial species, *Peptostreptococcus* sp. (NCTC 13430) and *Porphyromonas* sp. (NCTC 13428), were screened in co-cultures with health associated bacterial species *Neisseria* sp. (NCTC 13431) or *Frigovirgula* sp. (NCTC 13429). With the exception of *Neisseria* sp. (NCTC 13431) all speces were incubated independently for up to 4 days in fastidious anaerobic broth (FAB)(LabM, Lancs, UK) under anaerobic conditions at 37°C. *Neisseria* sp. (NCTC 13431) was incubated at 37°C for 16 h aerobically (statically in sealed bottles). Cells were harvested by centrifugation and resuspended in FAB to $OD_{600} = 0.5$.
Wells of a 96 well microtitre plate were inoculated with either: 200µl of each culture to be tested (as control); or 100µl of either *Neisseria* sp. (NCTC 13431) or *Frigovirgula* sp. (NCTC 13429) plus 100µl of either *Peptostreptococcus* sp. (NCTC 13430) or *Porphyromonas* sp. (NCTC 13428).
Plates were covered and incubated anaerobically for 24 h at 37° C. Co-culture assays were completed in triplicate.

Biomass

The total amount of biofilm grown on the 96 well plate was quantified using the crystal violet staining method as follows. For each well the liquid was removed and attached cell washed three times with TNMC (0.12g/l Tris, 20mg/l $MgCl_2$, 15mg/l $CaCl_2$ and 8.7g/l NaCl. Final solution adjusted to pH 8 with HCl). To each well 0.1 methanol was added and the plate incubated at room temperature for 15 mins, the methanol was aspirated off and the wells allowed to air dry. To each well 50 µl crystal violet 0.5% (w/v) was added for 1 min before washing wells once with 0.1 ml TNMC

and then four times with 0.2ml TNMC. To each well 100µl Acetic acid 0.7% (v/v) was added. After 15 min 0.1 ml of solution from each well was transferred to a new well plate and absorbance at 595 nm recorded. Biomass was represented as being directly proportional to the OD reading at 595nm (OD_{595}) of the samples compared to controls. Results were expressed as the reduction in OD_{595} seen in co-culture samples compared to monoculture controls, reflecting the effect of the active treatment on the amount of biofilm growth on the disc.

Statistical Analysis of Data

The data from each culture was analysed for statistical significance using a two tailed T- test (two sample assuming equal variance).

The results are shown in Figures 1 and 2. Total biomass was reduced in biofilms of *Porphyromonas* sp. (NCTC 13428) with each of *Frigovirgula* sp. NCTC 13429 and *Neisseria* sp. NCTC 13431 and in biofilms of *Peptostreptococcus* sp. (NCTC 13430) with each of *Frigovirgula* sp. NCTC 13429 and *Neisseria* sp. NCTC 13431. Total biomass was significantly reduced in biofilms of *Porphyromonas* sp. (NCTC 13428) in co-culture with *Neisseria* sp. (NCTC 13431) (P=0.0041) and in biofilms of *Peptostreptococcus* sp. (NCTC 13430) in co-culture with *Frigovirgula* sp. (NCTC 13429) (P= 0.0005).

Baac, H., J. P. Hajos, J. Lee, D. Kim, S. J. Kim, and M. L. Shuler. 2006. Antibody-based surface plasmon resonance detection of intact viral pathogen. Biotechnol. Bioeng. **94:**815-819.

Kohler, G. and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature **256:**495-497.

Lazcka, O., F. J. Del Campo, and F. X. Munoz. 2007. Pathogen detection: a perspective of traditional methods and biosensors. Biosens. Bioelectron. **22:**1205-1217.

Ngundi, M. M., N. V. Kulagina, G. P. Anderson, and C. R. Taitt. 2006. Nonantibody-based recognition: alternative molecules for detection of pathogens. Expert Rev. Proteomics **3:**511-524.

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO

MARS INCORPORATED,
6885 ELM STREET,
VIRGINIA 22101,
USA
[ATTN: DR. IAN J. DAVIS]
NAME AND ADDRESS
OF DEPOSITOR

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: *Porphyromonas* sp. IJD 1952	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCTC 13428

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☒ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This international depositary authority accepts the microorganism identified under I above, which was received by it on 25th July 2008 (date of the original deposit). [1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this international depositary authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NATIONAL COLLECTION OF TYPE CULTURES Address: HEALTH PROTECTION AGENCY, CENTRE FOR INFECTIONS, 61, COLINDALE AVENUE, LONDON NW9 5EQ UNITED KINGDOM	Signature(s) of person(s) having the power to represent the international depositary authority or of authorized official(s): Date: 23rd October 2008 Barry Holmes BARRY HOLMES HEAD OF NCTC

[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO MARS INCORPORATED, 6885 ELM STREET, VIRGINIA 22101, USA [ATTN: DR. IAN J. DAVIS] NAME AND ADDRESS OF DEPOSITOR	RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: *Frigovirgula* sp. IJD 1970	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCTC 13429

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☒ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This international depositary authority accepts the microorganism identified under I above, which was received by it on 25th July 2008 (date of the original deposit). [1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this international depositary authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

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[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

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INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Peptostreptococcus sp. IJD 1971	NCTC 13430

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☒ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This international depositary authority accepts the microorganism identified under I above, which was received by it on 25th July 2008 (date of the original deposit). [1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this international depositary authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NATIONAL COLLECTION OF TYPE CULTURES Address: HEALTH PROTECTION AGENCY, CENTRE FOR INFECTIONS, 61, COLINDALE AVENUE, LONDON NW9 5EQ UNITED KINGDOM	Signature(s) of person(s) having the power to represent the international depositary authority or of authorized official(s): Date: 23rd October 2008 Barry Holmes BARRY HOLMES HEAD OF NCTC

[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

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USA
[ATTN: DR. IAN J. DAVIS]
NAME AND ADDRESS
OF DEPOSITOR

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: *Neisseria* sp. IJD 1953	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCTC 13431

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☒ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This international depositary authority accepts the microorganism identified under I above, which was received by it on 4th September 2008 (date of the original deposit). [1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this international depositary authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NATIONAL COLLECTION OF TYPE CULTURES Address: HEALTH PROTECTION AGENCY, CENTRE FOR INFECTIONS, 61, COLINDALE AVENUE, LONDON NW9 5EQ UNITED KINGDOM	Signature(s) of person(s) having the power to represent the international depositary authority or of authorized official(s): Date: 17th November 2008 Barry Holmes BARRY HOLMES HEAD OF NCTC

[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

Claims

1. A canine foodstuff or oral care product which comprises a canine oral cavity probiotic.

2. A foodstuff, as claimed in claim 1, which is a kibble, wet food, semi-wet food, a chew or a drink.

3. An oral care product, as claimed in claim 1 which is a toothpaste, a gel or a mouthwash.

4. A foodstuff or product, as claimed in any one of claims 1 to 3, wherein the canine oral cavity probiotic is a *Frigovirgula* species or a *Neisseria* species.

5. A foodstuff or product, as claimed in claim 4, wherein the *Frigovirgula* species is *Frigovirgula patagoniensis*.

6. A foodstuff or product, as claimed in claim 4, wherein the *Neisseria* species is *Neisseria canis*.

7. A foodstuff or product, as claimed in claim 5 or claim 6, wherein the canine oral cavity probiotic is one or more of the strains deposited at the National Collection of Type Cultures, London, UK under the accession number NCTC 13429 or NCTC 13431.

8. A bacterial strain deposited at the National Collection of Type Cultures, London, UK under the accession number NCTC 13429 or NCTC 13431 or a bacterial strain which has a 16S rRNA gene sequence of 97% or more identical to the sequence set out in SEQ ID NO:1 or a bacterial strain which has a 16S rRNA gene sequence identical to the sequence set out in SEQ ID NO:2.

9. An antibody raised against a bacterial strain or part of a bacterial strain as

claimed in claim 8, or part of such an antibody.

10. An antibody which specifically binds a bacterial strain or part of a bacterial strain as claimed in claim 8 or part of such an antibody.

11. An antibody as claimed in claim 9 or claim 10 which is a monoclonal antibody or part of such an antibody.

12. Use of a bacterial strain or part of a bacterial strain as claimed in claim 8 in obtaining an antibody or part of such an antibody as claimed in any one of claims 9 to 11.

13. A composition comprising one or more of the bacterial strains as claimed in claim 8 and/or comprising an antibody or part of an antibody, as claimed in any one of claims 9 to 11.

14. A composition as claimed in claim 13 which is a foodstuff, an oral care product or a pharmaceutical.

15. A composition as claimed in claim 13 or claim 14 for use in medicine.

16. A composition as claimed in claim 15, for use in improving or maintaining canine oral health.

17. A method of detecting the presence of a bacterial strain as claimed in claim 8, by one or more of bacterial culture or by a detector for one or more of nucleic acid, peptide, carbohydrate or lipid or by amplification of nucleic acid of the micro-organism or by biochemical or phenotypic profiling.

18. A method, as claimed in claim 17, wherein the detector is an antibody or part of an antibody as claimed in any one of claims 9 to 11.

19. A kit for detecting the presence of the bacterial strain as claimed in claim 8, comprising an agent to determine the presence of the strain.

20. A kit, as claimed in claim 19, wherein the agent is an antibody as claimed in any one of claims 9 to 11.

21. A method of improving or maintaining canine oral health in a canine animal in need thereof, the method comprising administering to said canine animal a foodstuff, an oral care product or a pharmaceutical as claimed in any one of claims 1 to 7 or claim 14.

22. A bacterial strain deposited at the National Collection of Type Cultures, London, UK under the accession number NCTC 13430 or 13428 or a bacterial strain which has a 16S rRNA gene sequence identical to the sequence set out in SEQ ID NO:3 or SEQ ID NO:4.

23. Use of a bacterial strain, as claimed in claim 22, in identifying canine oral cavity probiotics.

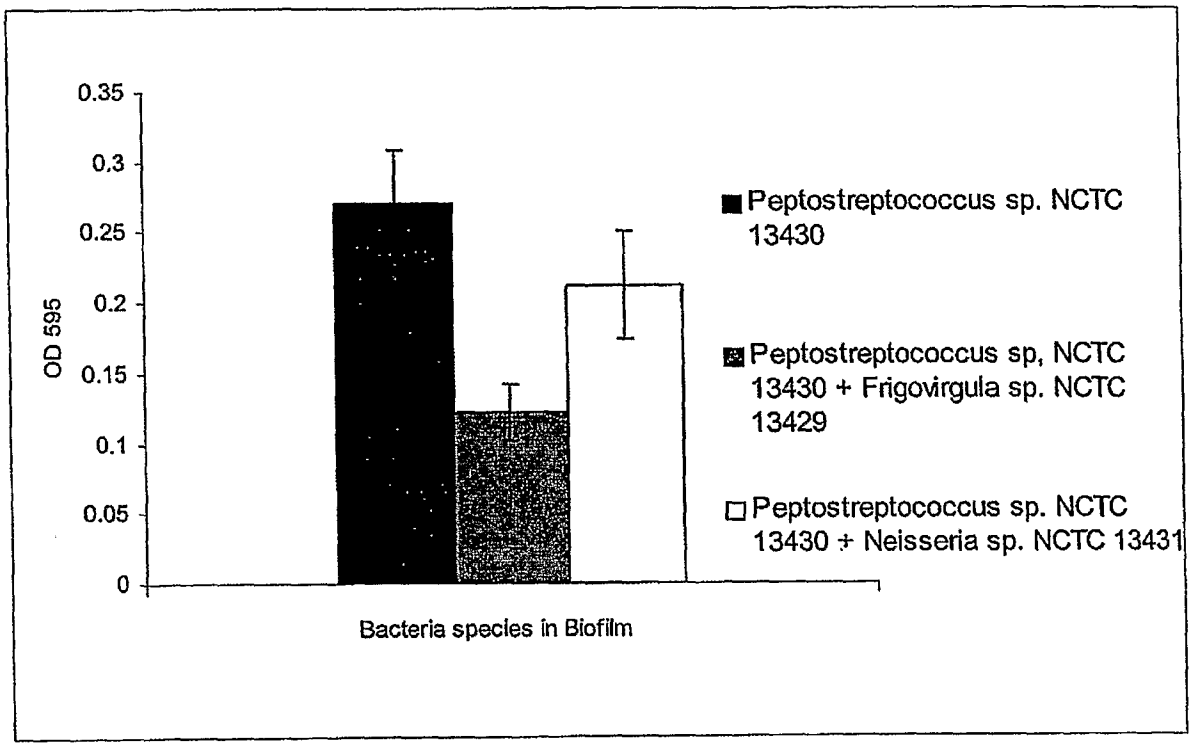
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
OD 595
Bacteria species in Biofilm
Peptostreptococcus sp. NCTC 13430
Peptostreptococcus sp, NCTC 13430 + Frigovirgula sp. NCTC 13429
Peptostreptococcus sp. NCTC 13430 + Neisseria sp. NCTC 13431

Figure 1.

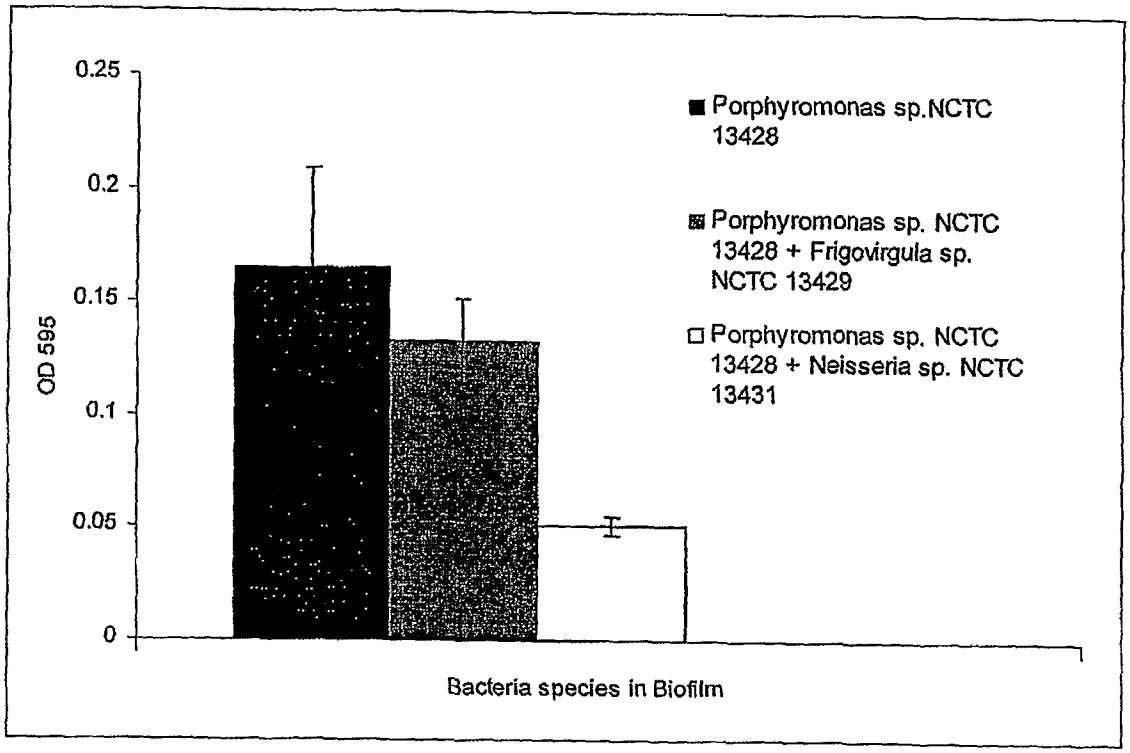
0.25
0.2
0.15
0.1
0.05
0
OD 595
Porphyromonas sp.NCTC 13428
Porphyromonas sp. NCTC 13428 + Frigovirgula sp. NCTC 13429
Porphyromonas sp. NCTC 13428 + Neisseria sp. NCTC 13431
Bacteria species in Biofilm

Figure 2.