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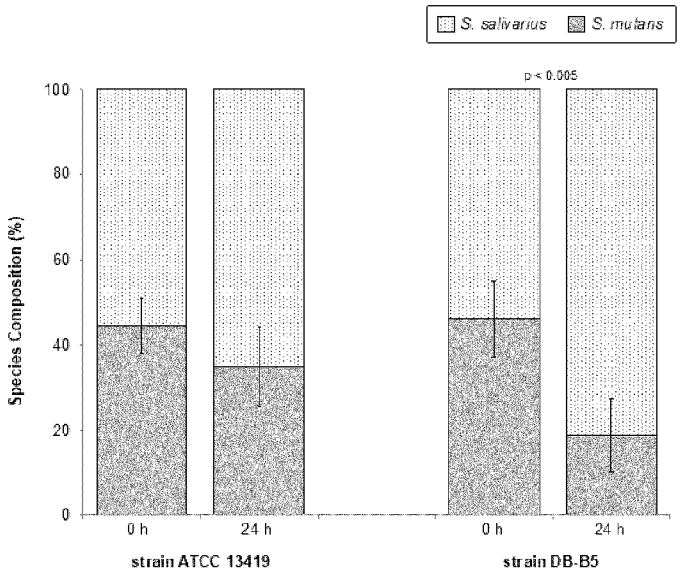
FIGURE 1



(57) Abstract: A novel strain of Streptococcus *salivarius* is provided herein which exhibits anti-microbial activity, and is, thus, useful to treat disease. The anti-microbial activity of the present *S. salivarius* strain, at least in part, is protease-resistant. The *S. salivarius* strain produces bacteriocins, including subtilosin. The *S. salivarius* strain advantageously inhibits oral pathogens, and thus, is useful to treat or prevent oral disease such as dental caries, periodontal disease (e.g. gingivitis and periodontitis), halitosis and dysbiosis within the oral cavity.

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[Continued on next page]

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PROBIOTIC FOR ORAL HEALTH

Field of the Invention

[0001] The present invention generally relates to the field of probiotics, and more particularly relates to a novel probiotic that promotes oral health.

Background of the Invention

[0002] The oral cavity houses one of the most diverse microbiotas in the human body. It provides several unique niches for bacterial colonization, such as the saliva, the tongue, the cheek, other mucosal surfaces, as well as the non-shedding surfaces of teeth. There are close to 800 unique oral bacterial species identified on the Human Oral Microbiome Database, with more species expected to be added with further sampling and identification. As with microbiota of other sites of the human body, a balanced oral microbiota is essential to the health of the human host. Alterations to this balance, or a dysbiotic microbial community, can not only lead to various diseases in the mouth, such as dental caries, periodontal disease, and halitosis, but can also cause disease systemically.

[0003] Dental caries is the most prevalent oral disease worldwide, estimated to have affected 2.4 billion people in 2016, while periodontal disease is the 11th most prevalent disease globally. Halitosis is a common problem in many individuals, and has a worldwide prevalence of 22% to 50%. Dental caries is caused by a combination of host factors and excessive dietary sugar intake, which leads to fermentation and production of acidic metabolites and extracellular polymeric substances by acidogenic bacteria residing in the supragingival plaque. Continued exposure to fermentable carbohydrates creates a positive feedback loop, where localized regions of low pH within the plaque biofilms produced by the acidogenic bacteria continues to select for acidogenic and aciduric bacteria. The continued exposure of the tooth to acidification causes the demineralization-remineralization balance to tilt towards demineralization, resulting in the progression of caries. *Streptococcus mutans* is the oral bacteria that is most associated with causing dental caries. Targeting *S. mutans* has been shown to be a successful method to reduce the incidence of dental caries.

[0004] Periodontal disease is an inflammatory disease induced by the subgingival microbiota, leading to the destruction of the supporting structures of the tooth. Unlike most dysbiotic diseases, periodontal disease is associated with increased microbial diversity in the subgingival crevice. Pathogenic species associated with periodontal disease include *Porphyromonas gingivalis, Tannerella forsythia*, *Fusobacterium nucleatum* and anaerobic species enriched in virulence factors that are able to

survive in the highly inflammatory environment of the diseased gum. When periodontal disease is severe, not only can it lead to tooth loss, but also to various systemic diseases such as atherosclerosis, adverse pregnancy outcomes, and rheumatoid arthritis.

[0005] For halitosis, or oral malodour, 80-90% of the cases are attributed to the volatile sulphur compounds (VSCs) released by the degradation of organic substances by oral anaerobic bacteria, mainly residing on the tongue. Some of the bacteria associated with periodontal disease, such as *Po. gingivalis*, are known VSC producers, and thus, periodontal disease is also associated with halitosis. Comparisons of tongue coatings of halitosis patients against healthy controls have revealed several VSC-producing bacterial species that are commonly found in halitosis patients. The genera *Prevotella* and *Leptotrichia* (including species *Pr. shahii, Pr. intermedia*, and *L. wadei*) are considered to be highly associated with halitosis, as well as other taxa such as *Peptostreptococcus stomatis, Capnocytophaga gingivalis* and *Ta. forsythia*.

[0006] Due to the recent awareness on the importance of maintaining a healthy microbiota, probiotics have become an exciting method for preventing or treating diseases associated with dysbiosis. Probiotics are defined by the Food and Agriculture Organization/World Health Organization (FAO/WHO) as "*live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host*". While the majority of probiotics developed are targeted to gut health, oral health probiotics are emerging as an important means to maintain the oral microbiota and prevent oral diseases.

[0007] Accordingly, it would be desirable to develop novel probiotics for use to promote oral health.

Summary of the Invention

[0008] A novel probiotic has now been developed that promotes oral health. Specifically, the probiotic is a novel strain of *Streptococcus salivarius*.

[0009] Thus, in one aspect of the invention, a novel strain of *Streptococcus salivarius* is provided which encodes for the bacteriocin, subtilosin.

[0010] In another aspect of the invention, a method of preventing disease in a mammal, or restoring oral health in mammal, is provided comprising administering a therapeutically effective amount of an *S. salivarius* probiotic that produces subtilosin to the oral cavity of the mammal.

[0011] In a further aspect of the invention, an *S. salivarius* strain is provided which exhibits anti-microbial activity that is protease-resistant.

[0012] These and other aspects of the invention are described in the detailed description by reference to the following figures.

Brief Description of the Invention

[0013] **Figure 1** graphically illustrates the results of a two-species growth competition assay performed against *S. salivarius* DB-B5 and *S. mutans* UA159, and against *S. salivarius* ATCC 13419 and *S. mutans* UA159;

[0014] **Figure 2** illustrates the phylogenetic reconstruction of *S. salivarius* DB-B5, including A) 16S rRNA Bayesian and Maximum Likelihood phylogenetic tree; and B) a multi-gene (dnaG, frr, infC, nusA, pgk, rplA, rpoB, rpsC, smpB, tsf) Bayesian and Maximum Likelihood phylogenetic tree. Bayesian posterior probabilities greater than 0.95 are shown at nodes, and branches with bootstrap support over 70% are thickened;

[0015] **Figure 3** provides the amino acid sequences of the coding regions within the subtilosin bacteriocin locus of the *S. salivarius* DB-B5 strain; and

[0016] **Figure 4** illustrates the full DNA sequence of the subtilosin bacteriocin locus of the *S. salivarius* DB-B5 strain. Coding regions are underlined, italic font indicates complement strand, bold font indicates forward strand and font with wave underline indicates overlapping start/stop regions.

Detailed Description of the Invention

[0017] A novel *Streptococcus salivarius* strain is provided which is useful to prevent and/or treat disease in a mammal. In one aspect, the present *S. salivarius* strain, herein referred to as *S. salivarius* DB-B5, encodes for the bacteriocin, subtilosin, inhibits multiple oral pathogens, and can colonize human cell surfaces.

[0018] The term "disease" is used herein to encompass disease within the oral cavity such as dental caries, periodontal disease (e.g. gingivitis and periodontitis), halitosis, dysbiosis within the oral cavity which can lead to systemic disease, as well as disease that occurs in other parts of the body, such as cancer or gastrointestinal disease by pathogenic microorganisms that originate in the oral cavity.

[0019] The present *S. salivarius* strain advantageously inhibits oral pathogens linked to disease. As used herein, the term "oral pathogen" refers to pathogenic microorganisms that exist in the oral cavity, or which originate in the oral cavity but may localize in other parts of the body to result in disease beyond the oral cavity. Examples of oral pathogens inhibited by the present *S. salivarius* strain include, but are not limited to, strains of the *Prevotella* genus such as *Pr. shahii,* strains of the *Porphyromonas* genus such as *Po. gingivalis*, strains of the *Fusobacterium* genus such as *F. nucleatum*, strains of the *Leptotrichia* genus such as *L. wadei*, and strains of the *Peptostreptococcus* genus such as *Pe. stomatis*.

[0020] The ability of an *S. salivarius* strain to inhibit pathogens may be determined using known assays such as an agar overlay assay in which a target pathogenic strain in liquid agar is added to an agar plate comprising homogeneous growth of the present *S. salivarius* strain. Detection of zones of inhibition, i.e. clear zones in which there is no growth of the target pathogen, indicates that the present *S. salivarius* strain inhibits the target pathogenic strain. The greater the zone (or width) of inhibition, the more virulent the inhibition.

[0021] The anti-pathogenic properties of the present *S. salivarius* strain are due, at least in part, to its bacteriocin expression profile. The present *S. salivarius* strain uniquely produces subtilosin, a bacteriocin which *S. salivarius* strains do not characteristically produce. The subtilosin locus of an *S. salivarius* strain according to an embodiment of the invention is shown in Figure 4, and the proteins it encodes are shown in Figure 3. Exemplary proteins encoded by this locus include an M13 family metallopeptidase, ABC transporter and ABC transporter ATP binding protein, radical S-adenosyl-L-methionine proteins, a helix-turn-helix domain-containing protein and phosphoglycerate mutase. Anti-pathogenic properties of the present *S. salivarius* strain are protease-resistant, at least in part, for example, these properties are resistant to proteases, such as at least one of pronase, subtilisin, or Proteinase K.

[0022] In addition, the present strain may encode other anti-pathogenic compounds, such as a thiazolyl peptide bacteriocin, not typically produced by *S. salivarius*, and a bacteriocin-like peptide (blp) bacteriocin. The bacteriocin loci may be found in the chromosomal DNA of the organism, or in extrachromosomal structures such as plasmids or episomes. The unique bacteriocin expression profile of the present *S. salivarius* strain provides the strain with inhibitory properties that allow it to target a broad range of pathogenic microorganisms.

[0023] In one embodiment, the present *S. salivarius* strain has a genome consisting of one circular chromosome (2,143,863 bp) with a GC content of 40.2%, one megaplasmid referred to as pIKMIN-B501 (138,497 bp) with a GC content of 35.6%, one small plasmid referred to as pIKMIN-B503 (3,225 bp) with a GC content of 39.6%, and one linear phage-like episome referred to as pIKMIN-B502 (57,714 bp) with a GC content of 39.1%. The strain possesses three separate loci encoding bacteriocins, a thiazolyl peptide (thiopeptide) locus on the megaplasmid, and subtilosin and *blpU* bacteriocin loci on the chromosome. The genome sequences were deposited at the National Centre for Biotechnology Information under GenBank accession numbers CP054153, CP054154, CP054155 and CP054156, the contents of which are incorporated herein by reference.

[0024] The present *S. salivarius* strain also advantageously exhibits significant growth dominance over the pathogen, *S. mutans*. When mixed and grown together under suitable growth

conditions, e.g. grown on BHI agar and incubated at 37 °C under 5% CO_2, the present *S. salivarius* strain outgrows *S. mutans* by at least about 10-90%, preferably by at least about 20%, 30% or 40%, for example, by about 40-80%.

[0025] The present *S. salivarius* strain is hydrophobic in nature, and thus, able to colonize human cell surfaces, enabling the strain to maintain a strong presence within the oral cavity on administration and thereby limit pathogen proliferation. The present *S. salivarius* strain exhibits a hydrophobicity of at least about 85%, preferably at least 90%-95%. As a comparison, other strains of *S. salivarius*, such as strain M18, exhibit a hydrophobicity of less than 80%. Hydrophobicity may be determined using assays established in the art, for example, the Microbial Adhesion to Hydrocarbon (MATH) assay which is based on the determination of microbial hydrophobicity by differential partitioning at an aqueous-hydrocarbon interface. The assay includes mixing of the bacterial cell suspension with a hydrocarbon, preferably an aliphatic hydrocarbon such as dodecane, hexadecane, octane or p-xylene, for a predetermined period to allow optimal interaction of the bacteria with the hydrocarbon phase. Partitioning of the bacterial suspension between the aqueous and hydrocarbon phases is determined, and expressed as the percentage of cells adsorbed by the hydrocarbon phase. Thus, surface hydrophobicity is based on the difference in absorbance of cells in suspension at 595 nm initially and following incubation with a hydrocarbon. Other methods to determine hydrophobicity may also be used such as the salt aggregation test in which bacterial cell suspensions are tested for visible aggregation or "salting out" in ammonium sulfate. Degree of hydrophobicity is determined using varying concentrations of ammonium sulfate (the greater the degree of aggregation at lower concentrations, the greater the hydrophobicity).

[0026] The present *S. salivarius* strain is physiologically acceptable for use as a probiotic for administration to the oral cavity. The present strain is susceptible to antibiotics such as Vancomycin (glycopeptides), Gentamicin (aminoglycosides), Streptomycin (aminoglycosides), Clindamycin (lincosamides), Erythromycin (macrolides), Ampicillin and Penicillin (penicillins), tetracycline and chloramphenicol (phenicols). The present strain has been determined to be a non-producer of toxic biogenic amines, such as histamine, tyramine, cadaverine, tryptamine, serotonin, putrescine and spermine/spermidine. In addition, the present strain does not encode virulent factors based on genome analysis that would render its use in mammals to be detrimental.

[0027] The present *S. salivarius* strain ferments carbohydrates. Exemplary carbohydrates fermented by the *S. salivarius* strain include, but are not limited to, D-galactose, D-glucose, fructose,

mannose, N-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, raffinose, gentiobiose, salicin, cellobiose, maltose, melibiose, saccharose, trehalose and inulin.

[0028] The present *S. salivarius* strain is useful to prevent and/or treat disease in a mammal. The term "mammal" is used herein to refer to both human and non-human mammals, including domesticated animals such as, but not limited to, cats, dogs and other pets, horses, livestock, etc. The method includes administering a therapeutically effective amount of the *S. salivarius* strain to a mammal to treat or prevent disease in the mammal. The term "therapeutically effective" refers to an amount of probiotic which is effective to reduce or minimize at least one factor associated with a target disease such as oral disease, including reducing the presence or activity of pathogenic microorganisms linked to dental caries such as *S. mutans*, pathogenic species associated with periodontal disease such as *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*, and volatile sulphur compound (VSC)-producing bacteria such as *Prevotella* (e.g. *Pr. shahii* and *Pr.intermedia*), *Leptotrichia* (e.g. *L. wadei*), *Peptostreptococcus stomatis and Capnocytophaga gingivalis,* and pathogenic species that may spread to cause disease elsewhere in the body, e.g. *Fusobacterium nucleatum*. In this regard, a therapeutically effective amount of the present *S. salivarius* strain is about 500 million to 20 billion CFUs per day.

[0029] The present *S. salivarius* strain may be formulated for administration in the form of a powder, tablet, capsule, suspension, and the like. Generally, probiotic cells such as the present *S. salivarius* cells, are dried to enhance viability/shelf-life, either by freeze-drying or spray-drying, using known techniques. The cells may be admixed with protective agents, such as, sweet whey concentrate, concentrated milk, lactose, saccharose, trehalose, galactose, starch, sorbitol, casein, beta-lactoglobulin, alpha- lactalbumin, soy, serum albumin, glutenin, prolamin, lysine, cysteine, glycine, or vitamins, to enhance survival following spray-drying. The dried cells may also be combined with one or more pharmaceutically acceptable excipients, i.e. which are not unacceptably toxic or otherwise unacceptable for administration to a mammal, such as a diluent, filler or bulking agent, lubricant, colorant, binder, coating agent, flavouring and/or sweetening agent, anti-caking agent, suppository base, etc. Suitable excipients are those which do not adversely affect *S. salivarius* growth and/or colonization. For powders, tablets and capsules, common excipients include, but are not limited to, microcrystalline cellulose (as binder/diluent), rice maltodextrin (as binder/diluent), mannitol or dextrin (filler), silicon dioxide (gliding/anti-caking agent), magnesium stearate (as lubricant), and hydroxy propyl methylcellulose (as suspending/viscosity agent).

[0030] The dried cells may be administered in dry form, or may be suspended in a liquid, e.g. an aqueous-based liquid, for oral administration, e.g. as an oral rinse, which may be ingested or discarded following rinsing of the oral cavity for a sufficient period of time, e.g. 30 seconds to 1 or 2 minutes.

[0031] The *S. salivarius* strain may also be formulated for administration in foods or beverages such as milk-based products, e.g. milk, yogurt, ice cream, puddings, whipped toppings or cheese products, drinks, fruit juices, soy or cereal-based products, confectionery products such as candy, gummies, chocolate, cookies, pastries and other baked goods, frosting and the like, and various other products. Chewing gums, lozenges, tooth paste or gel, aqueous gel and oral strips may also be prepared comprising the *S. salivarius* strain.

[0032] The present formulations may additionally include ingredients to enhance taste, including flavors, e.g. fruit or mint flavor, and/or sweetener, preferably non-fermentable sweetener such as aspartame, sucralose, stevia (steviol glycosides), and monk fruit extract, as well as carbohydrates that are metabolized via different pathways such as xylitol, erythritol, glycerol, and allulose.

[0033] As one of skill in the art will appreciate, the present strain may be combined with one or more additional probiotics, including those that also treat or prevent oral disease, and those which treat other ailments. Examples of such probiotics include other *S. salivarius* strains, e.g. K12 and M18 strains, other strains of Streptococcus species such as *S. thermophilus*, strains of Lactobacillus such as *L. reuteri, L. brevis, L. helveticus, L. plantarum, L. paracasei, L. casei, L. fermentum, L. rhamnosus,* and *L. acidophilus,* strains of Bifidobacterium such as *B. bifidum, B. longum, B. lactis, B. infantis*, and *B. breve,* and strains of Bacillus such as *B. subtilis* and *B. coagulans*.

[0034] *S. salivarius* formulations may be supplemented with one or more additional therapeutic agents or dietary supplements which do not adversely affect *S. salivarius* growth and/or colonization. Exemplary additives include, but are not limited to, fluoride, vitamins (e.g. vitamin D, vitamin E), minerals, prebiotics, agents that prevent cariogenic activity such as calcium phosphate salts, antioxidants and mixtures thereof.

[0035] The formulation may include a preservative, which does not adversely affect *S. salivarius* growth and/or colonization, such as essential oils (orange oil, grape oil, clove oil, etc.), benzoates, sorbates, and the like.

[0036] Embodiments of the invention are described by reference to the following specific examples which are not to be construed as limiting.

Example 1

[0037] The following methods were conducted to identify a novel strain of Streptococcus exhibiting properties having use in oral health.

[0038] *Bacterial Strains and Culture Conditions* - *Streptococcus salivarius* DB-B5 and other probiotic candidates were newly isolated from the supragingival plaque of a healthy female donor (see isolation procedure below). *Pr. shahii* DSM 15611, *F. nucleatum* DSM 15643, *Le. wadei* DSM 19758, and *Pe. stomatis* DSM 17678 were purchased from the DSMZ culture collection (German Collection of Microorganisms and Cell Cultures GmbH). *S. mutans* UA159, *S. salivarius* ATCC 13419 and *Po. gingivalis* ATCC 33277, were kindly provided by Dr. Cvitkovitch (University of Toronto, Faculty of Dentistry). All *Streptococcus* species were grown in Brain Heart Infusion (BHI; Hardy Diagnostics) at 37 °C in either anaerobic conditions, 5% CO_2, or in regular atmospheric conditions. *Po. gingivalis* ATCC 33277 and *Pr. shahii* DSM 15611, *F. nucleatum* DSM 15643, *Le. wadei* DSM 19758, and *Pe. stomatis* DSM17678 were grown in Schaedler's broth (Himedia) or plated on Brucella blood agar with hemin and vitamin K (Hardy Diagnostics) at 37 °C under anaerobic conditions. All anaerobic conditions were maintained using the Anaerogen atmosphere generation system (Oxoid).

[0039] *Isolation of Candidate Probiotic Strains* - Supragingival plaque was collected from a healthy female donor with no history of oral diseases and a strong family history of healthy teeth. A sterile toothpick was used to collect the plaque, and immediately placed in 500 µL of sterile phosphate-buffered saline (PBS; pH 7.0). The plaque was vortexed vigorously for 2 min, and filtered through a 5.0 µm filter to remove large debris and most eukaryotic cells. Serial dilutions were plated onto BHI (Hardy Diagnostics) agar and incubated anaerobically for 48 h at 37 °C. Colonies were picked and re-streaked onto fresh BHI agar plates, and incubated aerobically for 48 h at 37 °C. Surviving colonies were re-streaked an additional two times on BHI agar for purity.

[0040] *Antimicrobial Activity Against Oral Pathogens* - Probiotic candidates were assessed for their ability to inhibit oral bacteria associated with various oral diseases, using the bacterial inhibition overlay assay as described in Chew et al. (2015) J Appl Microbiol 118:1180–1190. https://doi.org/10.1111/jam.12772. The target bacteria used were: *Po. gingivalis* ATCC 33277, *Pr. shahii* DSM 15611, *F. nucleatum* DSM 15643, *L. wadei* DSM 19758 and *Pe. stomatis* DSM 17678. 10 µL of probiotic cells adjusted to OD_{595} = 1.0 were spotted onto BHI agar plates and incubated anaerobically or aerobically at 37 °C overnight. The target cells were each mixed with 8 mL of their respective growth media, supplemented with 0.7% agarose. The media was maintained at 40 °C to prevent solidification, and the target cells were added so that the final adjusted OD_{595} was 0.3-0.5. For

Pr. shahii, the final OD_{595} was adjusted to 0.01. The target cell suspension was poured onto the probiotic candidate spots, and allowed to solidify, prior to incubating in the required conditions for each target species. The zone of inhibition surrounding the probiotic candidate spots after incubation was measured.

[0041] *Competition Assay* - Bacterial growth competition was measured between an isolated probiotic strain (referred to herein as *S. salivarius* DB-B5) and *S. mutans* UA159, as well as between *S. salivarius* ATCC 13419 and *S. mutans* UA159, using quantitative PCR (qPCR). Fresh cells were harvested in BHI media, and competing strains were mixed 1:1 in microcentrifuge tubes (t = 0 h). 100 µL of the mixture was spread on BHI agar plates, and incubated for 24 h at 37 °C under 5% CO_2. After incubation, cells were harvested and DNA extraction was performed on both the 24 h harvest sample, as well as the 0 h sample. DNA extraction was performed using DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions for Gram-Positive Bacteria. qPCR was used to quantify the percent composition of the 2-species mixture, using the primers Ssaliv-qF and Ssaliv-qR for *S. salivarius* ATCC 13419, rpoB2-qF and rpoB2-qR for *S. salivarius* DB-B5, and Smut4-qF and Smut4-qR for *S. mutans* UA159 (Table 1).

Table 1. PCR and qPCR primers used in this study.

Primer Name	Sequence (5' - 3')	Target Gene
8F	AGAGTTTGATCCTGGCTCAG (SEQ ID NO:1)	16S rRNA
1492R	GGTTACCTTGTTACGACTT (SEQ ID NO:2)	
Ssaliv-qF	GTGTTGCCACATCTTCACTCGCTTCGG (SEQ ID NO:3)	gtfK
Ssaliv-qR	GTCAGCAGTAACTTCTGCTCGCTCT (SEQ ID NO:4)	
rpoB2-qF	TCGTCACGGTAACAAAGGGG (SEQ ID NO:5)	*rpoB*
rpoB2-qR	GGTCTTCTGAGCTTGCTCCAT (SEQ ID NO:6)	
Smut4-qF	AGGGTAGGCGATAGTGTCGAG (SEQ ID NO:7)	*rpoB*
Smut4-qR	TCATGTAAGCAACCACTGGATTT (SEQ ID NO:8)	

PowerUp SYBR Green Master Mix (Thermo Fisher Scientific) was used following the manufacturer's instructions, with an activation step of 2 min at 50 °C, an initial denaturation step of 2 min at 95 °C, 40 cycles consisting of 15 s at 95 °C, 15 s at 60 °C, and 45 s at 72 °C. The qTower^3-G (Analytik Jena) was used to run the qPCR and samples were analysed using the qPCRsoft 3.2 software. Ct values were calculated to colony forming units (CFU) using a standard curve of bacterial CFU ranging from 10^9 to 10^3 CFU for each species used.

[0042] *Molecular Identification of Candidate Probiotics* - Colony PCR was performed on the candidate probiotics to identify their species. Single colonies were diluted in 10 µL of sterile dH_2O to be used as the template. Phusion High-Fidelity PCR Kit (Thermo Fisher Scientific) was used following

the manufacturer's instructions, using the universal primers 8F and 1492R (Table 1), targeting the 16S rRNA gene. The cycle was run with an initial denaturation and cell breakage step of 1 min at 98 °C, 30 cycles consisting of 10 s at 98 °C, 15 s at 55 °C, and 45 s at 72 °C, followed by a final extension of 7 min at 72 °C. The PCR products were purified with QIAquick PCR purification kit (Qiagen) and sequenced using Sanger sequencing (The Centre for Applied Genomics, The Hospital for Sick Children) with the 8F and 1492R primers (Table 1). The resulting sequences were aligned and analyzed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the NCBI database.

[0043] *16S rRNA Phylogenetic Analysis* - 16S rRNA phylogeny reconstruction was performed as previously described (Naito et al. (2015). Proc Natl Acad Sci USA 112:7791–7796. https://doi.org/10.1073/pnas.1501676112). DNA was extracted from *S. salivarius* DB-B5 using DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's guide for Gram-Positive Bacteria. The DNA was used as a template for the amplification of 16S rRNA using the universal primers 8F and 1492R (Table 1). Phusion High-Fidelity PCR Kit (Thermo Fisher Scientific) was used following the manufacturer's instructions, with an initial denaturation time of 30 s at 98 °C, 30 cycles consisting of 10 s at 98 °C, 15 s at 55 °C, and 45 s at 72 °C, followed by a final extension of 7 min at 72 °C. The PCR product was purified with QIAquick PCR purification kit (Qiagen) and sequenced using Sanger sequencing (The Centre for Applied Genomics, The Hospital for Sick Children) with the 8F and 1492R primers (Table 1). The consensus 16S sequences were aligned against other publicly available 16S rRNA sequences obtained from the Integrated Microbial Genomes and Microbiomes database (IMG; https://img.jgi.doe.gov/), using MUSCLE (Edgar RC (2004). Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340). Bayesian and Maximum Likelihood phylogenies were reconstructed under the GTR+I+Γ model of nucleotide substitution using MrBayes v.3.2.6 (Ronquist et al. (2012). Syst Biol 61:539–542) run for 1,000,000 generations with 25% burn-in and PhyML (Guindon et al. (2010). Syst Biol 59:307–321) with 500 bootstrap replicates, respectively.

[0044] *Multi-gene Phylogenetic Analysis* - The multi-gene phylogenetic reconstruction was performed as previously described, with slight modifications (Naito et al. 2015). The amino acid sequences of the following genes, based on the Genomic Encyclopedia of Bacteria and Archaea were selected and concatenated: *dnaG, frr, infC, nusA, pgk, rplA, rpoB, rpsC, smpB, tsf.* The *S. salivarius* DB-B5 genes were obtained through Illumina sequencing of the full genome. Genes from all other bacterial species were obtained through IMG (https://img.jgi.doe.gov/). The concatenated amino acid sequences were aligned with MUSCLE. Bayesian and Maximum Likelihood phylogenies were

reconstructed under the WAG+I+Γ model of amino acid substitution using MrBayes v.3.2.6 run for 1,250,000 generations with 20% burn-in and PhyML with 500 bootstrap replicates, respectively.

[0045] *Carbohydrate Fermentation Profile* - To determine the carbohydrate metabolic capability of *S. salivarius* DB-B5, the API 50CH (bioMérieux) system was used, following the manufacturer's instructions. The API strip containing *S. salivarius* DB-B5 was incubated aerobically at 37 °C, and the fermentation profile was measured at both 24 h and 48 h.

[0046] *Cell Surface Hydrophobicity Assay* - The cell surface hydrophobicity was examined using the Microbial Adhesion to Hydrocarbon (MATH) assay, with slight modifications (as described in Zoueki et al. Journal of Colloid and Interface Science 344:492–496). Fresh *S. salivarius* DB-B5 cells were harvested and washed twice in PBS (pH 7.0). Cells were adjusted to an OD_{595} of 0.3 (A_0), and 2.5 mL of the cell suspension was placed in a glass tube. 450 µL of n-hexadecane (Sigma Aldrich) was added to the bacterial cell suspension, and vortexed for 2 min. The suspension was incubated at room temperature for 20 min to allow for phase separation. The aqueous phase was carefully removed and absorbance was measured at 595 nm (A_1). The surface hydrophobicity was calculated using the formula: Hydrophobicity (%) = $(A_0\text{-}A_1)/(A_0)$ x 100.

[0047] *Antibiotic Susceptibility Test* - Antibiotic susceptibility was measured using ETEST strips (bioMérieux), following the manufacturer's instructions. A lawn of *S. salivarius* DB-B5 was spread on a BHI agar plate, and a single ETEST strip was placed carefully on top using sterile forceps. The plates were incubated for 24 h at 37°C under 5% CO_2. The minimum inhibitory concentration (MIC) was measured by identifying the zone of inhibition that intersects the strip.

[0048] *Genome Mining for Antibiotic Resistance Genes using CARD* - The genome of *S. salivarius* DB-B5 was screened for genes involved in antibiotic resistance using the Comprehensive Antibiotic Resistance Database (CARD; https://card.mcmaster.ca/) (as described by Jia et al. (2017) Nucleic Acids Res 45:D566–D573). CARD is an online bioinformatic database of antibiotic resistance determinants organized through the Antibiotic Resistance Ontology (ARO). The protein sequences of all predicted open reading frames (ORF) of *S. salivarius* DB-B5 was used as input for the Resistance Gene Identifier (RGI) tool on the CARD website.

[0049] *Genome Mining for Virulence Factors using VFDB* - The Virulence Factor Database (VFDB)(Chen et al. (2016). Nucleic Acids Res 44:D694–D697) was used to screen for potential virulence factors in the *S. salivarius* DB-B5 genome. The VFDB core database was downloaded and a local reciprocal blastp analysis was performed against the protein sequences of all predicted ORFs of *S.*

salivarius DB-B5 using the BLAST+ software (Camacho et al. (2009). BMC Bioinformatics 10:421). Greater than 50% identity match and E-values of less than 10^{-5} were used as cut-off values.

Results

[0050] *Isolation of Oral Bacteria from Human Plaque* - To isolate candidate probiotic species for oral health, the supragingival plaque of a healthy female donor was collected. The female donor was chosen due to her strong dental history: a complete absence of oral diseases, including childhood caries, which is also shared by her family members. After filtration, diluted plaque was plated on BHI agar and grown for 48 h anaerobically. Colonies were re-streaked and grown for 48 h aerobically. Around 500 bacterial colonies that are facultative anaerobes were selected for further testing.

[0051] *Antimicrobial Activity against Oral Pathogens Associated with Periodontal Disease and Halitosis and their Molecular Identity* - Several target bacteria were chosen based on their association with various oral diseases: *Po. gingivalis* ATCC 33277 and *F. nucleatum* DSM 15643, as a target for periodontal diseases, and *Pr. shahii* DSM 15611, *Le. wadei* DSM 19758, and *Pe. stomatis* DSM 15611 as a target for halitosis. Based on the ability of isolates to inhibit at least two of the above target pathogens, 41 colonies were selected and their species identified via Sanger sequencing on their amplified 16S rRNA genes. The majority of the facultative anaerobe species were identified as *S. salivarius* or *Streptococcus oralis*. Table 2 shows the inhibition properties of the prime candidate probiotic, named *S. salivarius* DB-B5, and the common lab strain *S. salivarius* ATCC 13419 against the above pathogens. *S. salivarius* DB-B5 showed inhibitory properties against all of the pathogens, in differing amounts to the lab strain.

Table 2. Zone of inhibition (mm) of probiotic isolate *S. salivarius* DB-B5 and the common lab strain *S. salivarius* ATCC 13419 against oral pathogens.

	S. salivarius DB-B5	*S. salivarius* ATCC 13419
Po. gingivalis (periodontal, halitosis)	2.5	1.5
F. nucleatum (periodontal, halitosis)	4	4
Pr. shahii (halitosis)	6	1.5
L. wadeii (halitosis)	1	ND
Pe. stomatis (halitosis)	1	ND

*ND = not determined

[0052] *Competition Ability against S. mutans* - Since dental caries is one of the most common oral diseases, the ability of *S. salivarius* DB-B5 to outcompete *S. mutans* was measured. After 24 h of incubation, *S. salivarius* DB-B5 was able to significantly outcompete the caries-causing *S. mutans* UA159 (Fig. 1). Comparatively, the common lab strain, *S. salivarius* ATCC 13419, while showing slight

competition activity, was not able to significantly outcompete *S. mutans* UA159. This indicates that the candidate probiotic *S. salivarius* DB-B5 can reduce the levels of this caries-causing bacteria through competition.

[0053] *Phylogenetic Reconstruction of S. salivarius DB-B5* - In order to confirm the taxonomic placement of *S. salivarius* DB-B5, two different phylogenetic reconstructions were made. A 16S rRNA phylogenetic tree (Fig. 2A) and a multi-gene phylogenetic tree (Fig. 2B) using concatenated conserved genes based on the Genomic Encyclopedia of Bacteria and Archaea were constructed (see Wu et al. (2009). Nature 462:1056–1060). Both trees confirm the placement of the candidate probiotic as a novel strain of the *S. salivarius* species.

[0054] *Physical and Metabolic Characteristics of S. salivarius* DB-B5 – The carbohydrate fermentation profile of *S. salivarius* DB-B5 was determined and compared to *S. salivarius* K12 (Burton *et al.* (2006) *Appl Environ Microbiol* **72:** 3050-3), and other published *S. salivarius* phylotypes (P = phylotype, L = Lineage, I = Isolate) (van den Bogert *et al.* (2013) *FEMS Microbiol Ecol* **85:**376-88). Fermentation ability was measured with API 50CH strips (Biomérieux). "+" indicates the ability to ferment the carbohydrate substrate, "+/-" indicates weak or variable reaction, "-" indicates inability to ferment the substrate. *S. salivarius* DB-B5 was able to ferment 17 of the 49 carbohydrates tested. The comparison with other *S. salivarius* strains indicates that there are differences between the strains. The fermentation profile was found to be stable under numerous lab propagations, as well as fermentation and freeze-drying processes (data not shown).

Table 3. Carbohydrate fermentation profile of *S. salivarius* DB-B5 as compared to other S. salivarius strains

Substrate	DB-B5	K12	P3; L4; I1	P3; L5; I1	P3; L6; I1	P3; L6; I2	P3; L7; I1	P3; L7; I2
glycerol	-	-	-	-	-	-	-	-
erythritol	-	-	-	-	-	-	-	-
D-arabinose	-	-	-	-	-	-	-	-
L-arabinose	-	-	-	-	-	-	-	-
D-ribose	-	-	-	-	-	-	-	-
D-xylose	-	-	-	-	-	-	-	-
L-xylose	-	-	-	-	-	-	-	-
D-adonitol	-	-	-	-	-	-	-	-
methyl-βD-xylopyranoside	-	-	-	-	-	-	-	-
D-galactose	+	+	+	+	+/-	+/-	+	+/-
D-glucose	+	+	+	+	+/-	+/-	+	+
D-fructose	+	+	+	+	+/-	+/-	+	+
D-mannose	+	+	+/-	+/-	+/-	+/-	+	+/-
L-sorbose	-	-	-	-	-	-	-	-
L-rhamnose	-	-	-	-	-	-	-	-

dulcitol	-	-	-	-	-	-	-	-
inositol	-	-	-	-	-	-	-	-
D-mannitol	-	-	-	-	-	-	-	-
D-sorbitol	-	-	-	-	-	-	-	-
methyl-αD-mannopyranoside	-	-	-	-	-	-	-	-
methyl-αD-glucopyranoside	-	-	-	-	-	-	-	-
N-acetylglucosamine	+	+	+/-	-	-	-	+/-	-
amygdalin	+	-	-	-	-	-	-	-
arbutin	+	+	+	-	-	-	+	-
esculin ferric citrate	+	-	+	-	-	-	+	+
salicin	+	+	+	-	-	-	+	+/-
D-cellobiose	+	+	+/-	-	-	-	+/-	-
D-maltose	+	+	+	+	+	+/-	+	+
D-lactose (bovine origin)	-	+	+	+	+	+/-	+	+/-
D-melibiose	+	-	-	+/-	+	-	-	-
D-saccharose (sucrose)	+	+	+	+	+	+/-	+	+
D-trehalose	+	+	+	-	-	-	+	+
inulin	+	+	-	-	-	-	-	-
D-melezitose	-	-	-	-	-	-	-	-
D-raffinose	+	+	-	+/-	+/-	-	-	-
amidon (starch)	-	-	-	-	-	-	-	-
glycogen	-	-	-	-	-	-	-	-
xylitol	-	-	-	-	-	-	-	-
gentiobiose	+	-	-	-	-	-	-	-
D-turanose	-	-	-	-	-	-	-	-
D-lyxose	-	-	-	-	-	-	-	-
D-tagatose	-	+	-	-	-	-	-	-
D-fucose	-	-	-	-	-	-	-	-
L-fucose	-	-	-	-	-	-	-	-
D-arabitol	-	-	-	-	-	-	-	-
L-arabitol	-	-	-	-	-	-	-	-
potassium gluconate	-	-	-	-	-	-	-	-
potassium 2-ketogluconate	-	-	-	-	-	-	-	-
potassium 5-ketogluconate	-	-	-	-	-	-	-	-

[0055] The cell surface hydrophobicity of *S. salivarius* DB-B5 was also assessed, to determine the likelihood of the strain to be able to colonize human cell surfaces, such as the oral environment. Using the MATH assay, *S. salivarius* DB-B5's measured hydrophobicity was 98.70%. The high cell surface hydrophobicity indicates that the candidate probiotic is able to attach and colonize the human oral environment well.

[0056] *Safety Evaluation of S. salivarius DB-B5* - *S. salivarius* DB-B5 was assessed for its antibiotic susceptibility using Etest strips (bioMérieux). A diverse group of antibiotic classes were tested, and *S. salivarius* DB-B5 was found to be susceptible to all antibiotics tested, according to both the European (EFSA) and the U.S.'s (CLSI) breakpoints for *Streptococcus* species (Table 4). As a guide, the European Food Safety Authority (EFSA)'s MIC breakpoints for antimicrobial resistance of *Streptococcus thermophilus* (2018), and the Clinical and Laboratory Standards Institute (CLSI)'s MIC breakpoints for viridans Streptococci (2019) are listed. MIC values greater than or equal to breakpoints were considered resistant.

Table 4. Minimum inhibitory concentration (MIC) (μg/mL) of antibiotics against *S. salivarius* DB-B5.

	***S. salivarius* DB-B5**	**EFSA breakpoint**	**CLSI breakpoint**
Vancomycin (glycopeptide)	1	4	ND
Gentamicin (aminoglycoside)	6	32	ND
Streptomycin (aminoglycoside)	32	64	ND
Clindamycin (lincosamide)	0.064	2	1
Erythromycin (macrolide)	0.064	2	1
Ampicillin (penicillin)	0.125	2	8
Penicillin (penicillin)	0.19	ND	4
Tetracycline (tetracycline)	0.25	4	8
Chloramphenicol (phenicols)	2	4	16

[0057] Antibiotic resistance was further tested using *in silico* methods. All predicted ORFs from *S. salivarius* DB-B5 were run on the RGI tool from the CARD bioinformatic database, to detect potential antibiotic resistance genes. None of the ORFs from *S. salivarius* DB-B5 had "Perfect" (100% identical to the reference) or "Strict" (match bitscore above the curated BLASTP bitscore cutoff) hits against the database. RGI predicted 140 ORFs as "Loose" hits, which generally indicates distant homologs which may or may not have a role in antibiotic resistance.

[0058] The protein sequences of all predicted ORFs of *S. salivarius* DB-B5 were also analyzed for potential virulence genes using VFDB. The 15 VFDB-positive genes were compared to their actual predicted roles based on NCBI Blastp (nr database). The genes' presence in 7 commercially available probiotics were also assessed. The screened probiotics' genomes are *Bifidobacterium longum* 35624, *Lactobacillus helveticus* R0052, *Lactobacillus reuteri* SD2112/ATCC 55730, *Lactobacillus rhamnosus* GG, *L. rhamnosus* R0011, *S. salivarius* K12, and *S. salivarius* M18. There were 15 hits in total, and these genes were further analyzed for their virulence potential, along with their presence in 7 commercially available probiotic strains with publicly available genomes (Table 5). As seen in Table 5,

the 15 genes are commonly found in many bacteria, including most of the commercially available probiotic strains with GRAS status. The genes are not believed to be of concern, with respect to safety.

Table 5. Analysis of *S. salivarius* DB-B5 genes with hits to VFDB.

DB-B5 Locus Tag	BLAST hit (nr)	BLAST E-value (%ID)	VFDB gene hit	VFDB E-value (%ID)	Found in Pro-biotics	Analysis
HRE60_08810	UTP-glucose-1-phosphate uridylyltransferase [*S. salivarius*]	0 (100%)	UDP-glucose pyrophosphorylase [Hyaluronic acid capsule - *S. pyogenes*]	0 (88.7%)	yes (7/7)	Hyaluronic acid capsule is a virulence factor in Group A Strep only. This gene is found in most bacteria for glycogenesis and cell wall metabolism. Other genes of hyaluronic acid capsule biosynthesis are not present.
HRE60_06080	peptide-methionine (R)-S-oxide reductase [*S. salivarius*]	0 (97.2%)	trifunctional thioredoxin/methionine sulfoxide reductase [*N. meningitidis*]	3.89E-131 (55.8%)	yes (7/7)	Normal stress-related protein found in most bacteria.
HRE60_05360	N-acetylmuramidase [*Streptococcus* sp.]	8.78E-170 (99.2%)	autolysin [*L. monocytogenes*]	2.26E-40 (51.0%)	yes (6/7)	Normal hydrolase of peptidoglycan. Conserved domain is different from autolysin of *L. monocytogenes*.
HRE60_05035	DUF814 domain-containing protein [*S. salivarius*]	0 (99.5%)	fibronectin-binding protein [*S. pyogenes*]	0 (76.9%)	yes (6/7)	Normal component of the ribosome quality control complex that binds fibronectin/fibrinogen.
HRE60_04280	UDP-glucose 4-epimerase GalE [*Streptococcus* sp.]	0 (100%)	UDP-glucose 4-epimerase [LOS - *H. influenzae*]	1.19E-153 (60.6%)	yes (7/7)	Found in all bacteria - epimerase for galactose and glucose. Also used in LPS/LOS biosynthesis, n/a in *Streptococcus* spp.
HRE60_03525	metal ABC transporter substrate-binding [*S. salivarius*]	0 (99.7%)	Mn-binding adhesion; Mn ABC transporter [*S. pneumoniae*]	0 (81.5%)	yes (7/7)	Found in most bacteria. The protein may act as an adhesin.
HRE60_01785	beta-hydroxyacyl-ACP dehydratase [*Streptococcus* sp.]	7.03E-98 (100%)	(3R)-hydroxymyristoyl ACP dehydratase [LPS - *B. melitensis*]	1.36E-42 (50.8%)	yes (6/7)	Part of normal fatty acid biosynthesis in bacteria. Not related to virulence in *Streptococcus*.
HRE60_02780	Clp protease ATP-binding subunit [*S. salivarius*]	0 (99.4%)	Clp protease [*L. monocytogenes*]	0 (60.0%)	yes (7/7)	Heat shock protein found in most bacteria. Clp protease is important in *L. monocytogenes'* intracellular survival. No sign of contribution to virulence in non-intracellular probiotics species.
HRE60_01580	Clp protease proteolytic subunit [*Streptococcus* sp.]	1.14E-142 (100%)	Clp protease proteolytic subunit [*L. monocytogenes*]	3.16E-91 (63.5%)	yes (7/7)	
HRE60_04615	UDP-galactopyranose mutase [*S. salivarius*]	0 (100%)	UDP-galactopyranose mutase [*E. faecalis*]	3.95E-170 (62.8%)	yes (4/7)	Genes found in most bacteria for phospholipid metabolism.
HRE60_00955	phosphatidate cytidylyltransferase [*Streptococcus* sp.]	0 (99.6%)	phosphatidate cytidylyltransferase [*E. faecalis*]	1.03E-88 (51.7%)	yes (7/7)	
HRE60_04575	sugar transferase [*S. salivarius*]	0 (99.8%)	glycosyl transferase CpsE [Capsule - *S. agalactiae*]	1.66E-163 (50.4%)	yes (7/7)	Cps genes in *S. agalactiae* is involved in the biosynthesis of type III capsular polysaccharide, considered virulent only in Group B Strep.
HRE60_04570	tyrosine protein kinase [*S. salivarius*]	0 (100%)	CpsD autokinase [Capsule - *S. agalactiae*]	1.83E-99 (61.3%)	yes (7/7)	
HRE60_04560	tyrosine protein phosphatase [*S. salivarius*]	0 (99.6%)	CpsB phosphatase [Capsule - *S. agalactiae*]	1.48E-130 (71.6%)	yes (6/7)	
HRE60_00985	chaperonin GroEL [*Streptococcus* sp.]	0 (100%)	Hsp60 heat shock protein [*L. pneumophila*]	0 (58.1%)	yes (7/7)	Found in most bacteria. Not related to virulence in *Streptococcus*.

Discussion

[0059] Probiotics have the ability to prevent disease while maintaining or restoring a healthy microbiota, and thus have become an attractive method as a preventative treatment in oral diseases. A major beneficial action of probiotics is their ability to protect the host against pathogen colonization. This study has indicated that at least two independent methods of protecting against pathogens exists in *S. salivarius* DB-B5: the secretion of an antimicrobial molecule that actively kills oral pathogens, including *Po. gingivalis, Pr. shahii, F. nucleatum, L. wadei,* and *Pe. stomatis,* and a mechanism that allows *S. salivarius* DB-B5 to outcompete *S. mutans* and inhibit its growth when grown together.

[0060] *S. salivarius* DB-B5 showed an extremely high cell surface hydrophobicity of 98.70%. This is one of the highest percentages reported amongst probiotic strains. A high cell surface hydrophobicity is used as a proxy for the bacteria's ability to be able to colonize the host, and thus, is a desirable trait in probiotics. In the case of probiotics for oral health, the probiotics must adhere to various surfaces inside the mouth. Depending on the mode of delivery, the oral probiotics will have less time to colonize the oral environment, compared to the time gut probiotics have to colonize the gastrointestinal tract. Thus, possessing a high cell surface hydrophobicity is a desirable characteristic in oral probiotics.

[0061] Extensive *in vitro* and *in silico* safety analysis of *S. salivarius* DB-B5 was conducted to ensure its suitability for human consumption. Generally, *S. salivarius* is considered a safe species since it is a normal commensal of the oral microbiota, acquired immediately after birth.

[0062] Antibiotic resistance and the horizontal transfer of resistance genes is one of the most important crises facing the medical community today. The susceptibility of probiotics to antibiotics is essential if it is to be widely used by consumers. *S. salivarius* DB-B5 was shown to be susceptible to the various antibiotics tested, which spanned a wide-range of antibiotic classes. The breakpoints to indicate susceptibility was based on the European Food Safety Authority (EFSA), as well as the Clinical and Laboratory Standards Institute (CLSI) documents. In order to ensure that *S. salivarius* DB-B5 does not have any antibiotic resistance genes that may become mobile, the whole genome was mined for antibiotic resistance genes *in silico*. There was a good correspondence between the genotype and the phenotype for antibiotic resistance, where only loose hits (considered distant homologs which may or may not have a role in antibiotic resistance) were detected. This indicates that *S. salivarius* DB-B5 is safe to use commercially, with regards to antibiotic resistance.

[0063] 15 VFDB-positive genes were analyzed for their potential roles as virulence factors. All of the genes analyzed were common genes found in most bacteria, such as heat shock proteins, or genes

involved in the biosynthesis of surface structures. Some of the genes flagged by VFDB are part of gene clusters involved in the biosynthesis of virulence factors only in specific taxa. For instance, a gene identified as UDP-glucose pyrophosphorylase by VFDB is involved in hyaluronic acid capsule biosynthesis in Group A Streptococcus such as *Streptococcus pyogenes*. However, the same gene has a better hit with UTP-glucose-1-phosphate uridylyltransferase according to the nr database on NCBI BLAST, which is required for glycogenesis and cell wall metabolism in most bacteria. Furthermore, all other genes involved in hyaluronic acid biosynthesis were absent in the *S. salivarius* DB-B5 genome. Further analysis of all other genes that were positively identified under VFDB were found to have no actual virulence potential in *S. salivarius* DB-B5. The VFDB-positive genes were also present in all or most of the commercially available probiotics tested (both gut and oral), based on their genome. Overall, *S. salivarius* DB-B5 seems to be safe as a candidate probiotic based on both *in vitro* and *in silico* results.

[0064] Several clinical trials have been performed on the ability of probiotics to prevent or treat oral diseases. *Lactobacillus rhamnosus* L8020 was used to ferment a yogurt product, which showed significant decrease in oral pathogen levels, compared to a placebo yogurt [1]. A combination of *L. rhamnosus* GG and *Bifidobacterium lactis* BB-12, normally used as gut probiotics, was delivered in lozenge format to healthy participants, and was shown to be able to reduce plaque index, gingival index, and decrease levels of gingival pathogens [2]. Several clinical trials using *Lactobacillus salivarius* WB21-containing tablets have been performed, and the probiotic was shown to decrease periodontal conditions, periodontal pathogens, halitosis, and halitosis-associated pathogens [3–5]. *S. salivarius* K12 has been shown to reduce halitosis levels when used in conjunction with chlorhexidine, while *S. salivarius* M18 usage was shown to reduce cariogram outcome in at-risk children [6,7]. The above trials indicate that oral probiotics are able to prevent and treat a wide-range of oral diseases, using a variety of delivery formats. The results of this study, thus, indicates that the present oral probiotic strain that can tackle three prevalent oral diseases, e.g. dental caries, periodontal disease, and halitosis.

Example 2 – Sequence analysis of *S. salivarius* DB-B5

[0065] In order to further analyze the present *S. salivarius* strain, whole-genome sequencing was performed.

[0066] *S. salivarius* DB-B5 was grown on BHI media (Hardy Diagnostics) at 37 °C under 5% CO_2. Cells were harvested, and genomic DNA extracted using DNeasy Blood & Tissue Kit (Qiagen). Paired-end sequencing libraries (2 x 300 bp) were constructed using the Nextera XT Library Kit (Illumina) and sequenced on the MiSeq v3 platform (Illumina) at the Centre for the Analysis of Genome Evolution and Function (CAGEF) at the University of Toronto. In parallel, DB-B5 genomic DNA was

also extracted using the Wizard Genomic DNA Purification Kit (Promega) and sent to Génome Québec for large insert library preparation and PacBio Sequel sequencing. Illumina reads were quality filtered using Trimmomatic (v0.38.0) (8). Unicycler (v0.4.8.0) was used to perform a *de novo* hybrid assembly with 1,722,228 Illumina paired-end reads and 629,563 PacBio long reads, with a genome coverage of ~1000x (9). The complete genome consists of one circular chromosome (2,143,863 bp) with a GC content of 40.2%, one megaplasmid named pIKMIN-B501 (138,497 bp) with a GC content of 35.6%, one small plasmid named pIKMIN-B503 (3,225 bp) with a GC content of 39.6%, and one linear phage-like episome named pIKMIN-B502 (57,714 bp) with a GC content of 39.1%. The genome was annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4.11 (10). The genome contains a total of 2,041 protein coding genes, 18 complete rRNA genes, 4 non-coding RNA genes (ncRNA), and 68 tRNA genes.

[0067] A search of the genome of *S. salivarius* DB-B5 was conducted to identify bacteriocins using BAGEL4 (http://bagel4.molgenrug.nl/), a web-based genome mining tool for bacteriocins and RiPPs (ribosomally synthesized and post-translationally modified peptides) (11). The analysis revealed that the strain possesses a thiazolyl peptide (thiopeptide) bacteriocin locus on the megaplasmid pIKMIN-B501, and a *blpU* bacteriocin locus on the chromosome. The genome sequence of *S. salivarius* DB-B5 will be useful in further understanding the mechanism of its probiotic properties.

[0068] The complete genome sequences have been deposited at GenBank under the accession numbers CP054153 (chromosome), CP054154 (pIKMIN-B501), CP054155 (pIKMIN-B502), and CP054156 (pIKMIN-B503).

[0069] The *S. salivarius* DB-B5 strain was deposited at The International Depository Authority of Canada (IDAC) under accession no. 160720-1 (depositor reference, DOSEBIOSYSTEMS-SS_DBB5), on July 16, 2020, in accordance with the Budapest Treaty.

Example 3 – Anti-microbial Activity

[0070] Overnight cultures of DB-B5 were filter sterilized and the resulting supernatant was passed through a column containing XAD-16 resin. The resin was then washed with 50% methanol and the antimicrobial activity was eluted with a 95% methanol wash to create a crude extract. The crude extract was further purified using a C18 clean-up column and antimicrobial activity was eluted with a 60% methanol elution. The methanol was removed via evaporation and the sample was resuspended in phosphate buffer (pH =6.5).

[0071] To 100 ul of DB-B5 extract cleaned as described above, proteases (pronase, subtilisin, or Proteinase K) were added at a 1mg/mL concentration and various halitosis-causing bacterial cells were incubated in the protease-containing extract for 2 hours at 37 °C. A control with no protease was also incubated with microbial cells. To all samples was added 10 ul of 10x TRIS, CaCl buffer. Protease only controls were also included (protease, 10 ul of 10x Tris, and 100 ul of phosphate buffer), as well as a heat-inactivated sample (100 ul of DB-B5 extract, boiled at 100 °C for 30 minutes).

[0072] Soft agar plates were prepared with Schadler's broth and were allowed to harden. 10 ul of each sample were spotted onto a plate, dried and incubated at 37 °C anaerobically overnight. The DB-B5 extract exhibited inhibition of all bacteria tested in the presence of proteases, indicating that the antimicrobial activity was not protein-dependent as shown in the Table 6 below.

Table 6.

Antimicrobial Activity Assays								
Halitosis Bacteria	Crude Extract	Protease Controls			Crude Extracts + Treatment			
		Proteinase K	Pronase	Subtilisin	Proteinase K	Pronase	Subtilisin	30 minute 100C
Peptostreptococcus stomatis	+	-	-	-	+	+	+	+
Porphyromonas gingivalis	+	-	-	-	+	+	+	+
Solobacterium moorei	+	-	-	-	+	+	+	+
Tannerella forsythia	+	-	-	-	+	+	+	+
Treponema denticola	+	-	-	-	+	+	+	+
Leptotrichia wadei	+	+	-	-	+	+	+	+
Fusobacterium nucleatum	+	-	-	-	+	+	+	+
Prevotella shahii	+	+	-	-	+	+	+	+

* a + indicates a zone of clearing in a soft agar spot assay
* a - indicates no zone of clearing in a soft agar spot assay
* All proteases were used at a final concentration of 1mg/ml

[0073] Thus, the active antimicrobial compound against these halitosis-causing bacteria is protease-resistant.

Example 4 - Biogenic Amines (BA) Production

[0074] *S. salivarius* DB-B5 was tested for the ability to produce BAs using both an *in silico* method to detect the presence of genes required for BA production, as well as an *in vitro* plating method to detect the presence of the BA. *In silico* results indicated that the genes involved in BA production were not present in the *S. salivarius* DB-B5 genome as shown in Table 7.

Table 7.

BA	Enzyme for BA Production	Presence in DB-B5
histamine	histidine decarboxylase	no
tyramine	tyrosine decarboxylase	no
cadaverine	lysine decarboxylase	no
tryptamine	tryptophan decarboxylase	no
serotonin	hydroxytryptophan decarboxylase	no
putrescine	ornithine decarboxylase	no
spermine/spermidine	spermine synthase, spermidine synthase, carboxynorspermidine synthase + decarboxylase	no

[0075] The *in vitro* plating analysis also confirmed this result: *S. salivarius* DB-B5 and *E. coli* ATCC 31616 were streaked on decarboxylase media containing different amino acid precursors. *S. salivarius* growth did not cause a pH color change, whereas *E. coli* growth caused the decarboxylase media to change to a distinct purple color, indicating the rise of pH to more alkaline conditions due to the presence of biogenic amines.

Example 5 - Clinical Study on halitosis

[0076] In a randomized, double-blind, placebo-controlled study in healthy volunteers, the ability of *S. salivarius* DB-B5 to reduce halitosis caused by oral bacteria was determined by measuring hydrogen sulfide and methyl mercaptan levels.

[0077] Participants consumed DB-B5 powder dissolved in 4 oz of water twice a day (2 billion CFU/day or 10 billion CFU/day). The DB-B5 water was swirled around in the mouth for 15 seconds prior to ingestion. Participants took DB-B5 water twice daily, for 4 weeks.

[0078] Hydrogen sulfide and methyl mercaptan levels were measured at baseline and 4 weeks using an OralChroma device. Hydrogen sulfide is a volatile sulfur compound that is associated with halitosis-causing bacteria residing on the dorsum of the tongue, and accounts for about 90% of the cause of halitosis. Methyl mercaptan is a volatile sulfur compound that is associated with periodontal disease-causing bacteria. DB-B5 was able to reduce the levels of hydrogen sulfide in the mouth of healthy participants by about 50%, as well as reduced levels of methyl mercaptan as shown in Table 8.

Table 8.

Intervention Group	Hydrogen Sulfide			Methyl Mercaptan		
	2 billion CFU/day	10 billion CFU/day	placebo	2 billion CFU/day	10 billion CFU/day	placebo
Baseline Mean Score (ppb)	450	563	506	175	194	149
Week 4 Mean Score (ppb)	218	309	518	85	112	143
Mean Change from Baseline[1] (ppb)	232	254	-12	90	82	6
Standard Deviation of Mean Change Score (ppb)	240	156	83	405	311	84
Percent Change[1] (%)	52	45	-2	51	42	4
P-value vs. placebo	0.043	0.026	-	0.276	0.311	-
Direction of Change (week 4 vs baseline)	↓	↓	-	↓	↓	-

[1]Positive numbers indicates a lower value at week 4 compared to baseline. Negative numbers indicates a higher value at week 4 compared to baseline.

[0079] Relevant portions of references referred to herein are incorporated by reference.

References:

1. Nikawa H, Tomiyama Y, Hiramatsu M, et al (2011) Bovine milk fermented with Lactobacillus rhamnosus L8020 decreases the oral carriage of mutans streptococci and the burden of periodontal pathogens. J Investig Clin Dent 2:187–196. https://doi.org/10.1111/j.2041-1626.2011.00056.x

2. Toiviainen A, Jalasvuori H, Lahti E, et al (2015) Impact of orally administered lozenges with Lactobacillus rhamnosus GG and Bifidobacterium animalis subsp. lactis BB-12 on the number of salivary mutans streptococci, amount of plaque, gingival inflammation and the oral microbiome in healthy adults. Clin Oral Investig 19:77–83. https://doi.org/10.1007/s00784-014-1221-6

3. Suzuki N, Yoneda M, Tanabe K, et al (2014) Lactobacillus salivarius WB21--containing tablets for the treatment of oral malodor: a double-blind, randomized, placebo-controlled crossover trial. Oral Surg Oral Med Oral Pathol Oral Radiol 117:462–470. https://doi.org/10.1016/j.oooo.2013.12.400

4. Mayanagi G, Kimura M, Nakaya S, et al (2009) Probiotic effects of orally administered Lactobacillus salivarius WB21-containing tablets on periodontopathic bacteria: a double-blinded, placebo-controlled, randomized clinical trial. J Clin Periodontol 36:506–513. https://doi.org/10.1111/j.1600-051X.2009.01392.x

5. Shimauchi H, Mayanagi G, Nakaya S, et al (2008) Improvement of periodontal condition by probiotics with Lactobacillus salivarius WB21: a randomized, double-blind, placebo-controlled study. J Clin Periodontol 35:897–905. https://doi.org/10.1111/j.1600-051X.2008.01306.x

6. Jamali Z, Aminabadi NA, Samiei M, et al (2016) Impact of Chlorhexidine Pretreatment Followed by Probiotic Streptococcus salivarius Strain K12 on Halitosis in Children: A Randomised Controlled Clinical Trial. Oral Health Prev Dent 14:305–313. https://doi.org/10.3290/j.ohpd.a36521

7. Di Pierro F, Zanvit A, Nobili P, et al (2015) Cariogram outcome after 90 days of oral treatment with Streptococcus salivarius M18 in children at high risk for dental caries: results of a randomized, controlled study. Clin Cosmet Investig Dent 7:107–113. https://doi.org/10.2147/CCIDE.S93066

CLAIMS

1. A strain of *Streptococcus salivarius* that encodes for the bacteriocin, subtilosin.

2. The *S. salivarius* strain of claim 1, which additionally encodes for a thiazolyl peptide and/or a bacteriocin-like peptide (blp) bacteriocin.

3. The *S. salivarius* strain of claim 1, which exhibits the following properties:
   i) inhibits oral pathogens;
   ii) outcompetes *S. mutans*;
   iii) is able to colonize human cell surfaces;
   iv) is susceptible to antibiotics; and/or
   v) is a non-producer of biogenic amines.

4. The *S. salivarius* strain of claim 1, which inhibits the following oral pathogens: *Po. gingivalis, Ta. forsythia, Pr. shahii, F. nucleatum, L. wadei,* and *Pe. Stomatis.*

5. The *S. salivarius* strain of claim 1, which exhibits a cell-surface hydrophobicity of at least about 85%.

6. The *S. salivarius* strain of claim 1, comprising the bacteriocin locus of SEQ ID NO: 19.

7. The strain of claim 1, comprising the following genome sequences:
   i) chromosomal sequence of GenBank accession no. CP054153;
   ii) megaplasmid sequence of GenBank accession no. CP054154;
   iii) episome sequence of GenBank accession no. CP054155; and
   iv) plasmid sequence of GenBank accession no. CP054156.

8. The *S. salivarius* strain of claim 1, which exhibits protease-resistant anti-microbial activity.

9. The *S. salivarius* strain of claim 8, wherein the anti-microbial activity is resistant to at least one of pronase, subtilisin, or Proteinase K.

10. A method of treating or preventing disease in a mammal comprising administering a therapeutically effective amount of an *S. salivarius* strain as defined in claim 1 to the mammal.

11. The method of claim 10, wherein the *S. salivarius* strain is combined with pharmaceutically acceptable excipients for oral administration.

12. The method of claim 10, wherein the *S. salivarius* strain is administered in an amount of 500 million to 20 billion CFUs per day.

13. The method of claim 10, wherein the *S. salivarius* strain is combined with one or more probiotics.

14. The method of claim 10, wherein the *S. salivarius* strain is administered to the oral cavity.

15. The method of claim 10, comprising the additional step of maintaining the *S. salivarius* strain in the oral cavity for a period of time prior to ingesting or expectorating.

16. The method of claim 10, wherein the disease is an oral disease selected from the group consisting of dental caries, periodontal disease, gingivitis, halitosis and dysbiosis

17. A composition comprising an *S. salivarius* strain as defined in claim 1 combined with one or more pharmaceutically acceptable excipients.

18. A composition comprising an *S. salivarius* strain as defined in claim 1 combined with one or more probiotics.

19. A composition comprising an *S. salivarius* strain as defined in claim 1 combined with a food or beverage.

20. A composition comprising an *S. salivarius* strain as defined in claim 1 formulated for oral administration.

21. The composition of claim 18, wherein the composition is a rinse, paste, gel or lozenge.

22. A *Streptococcus salivarius* strain which is deposited at The International Depository Authority of Canada (IDAC) under accession no. 160720-1.

23. A *Streptococcus salivarius* strain which exhibits anti-microbial activity that is protease-resistant.

24. The *S. salivarius* strain of claim 23, wherein the anti-microbial activity is resistant to at least one of pronase, subtilisin, or Proteinase K.

25. The *S. salivarius* strain of claim 23, which exhibits the following properties:

i) inhibits oral pathogens;

ii) outcompetes *S. mutans*;

iii) is able to colonize human cell surfaces;

iv) is susceptible to antibiotics; and/or

v) is a non-producer of biogenic amines.

FIGURE 1

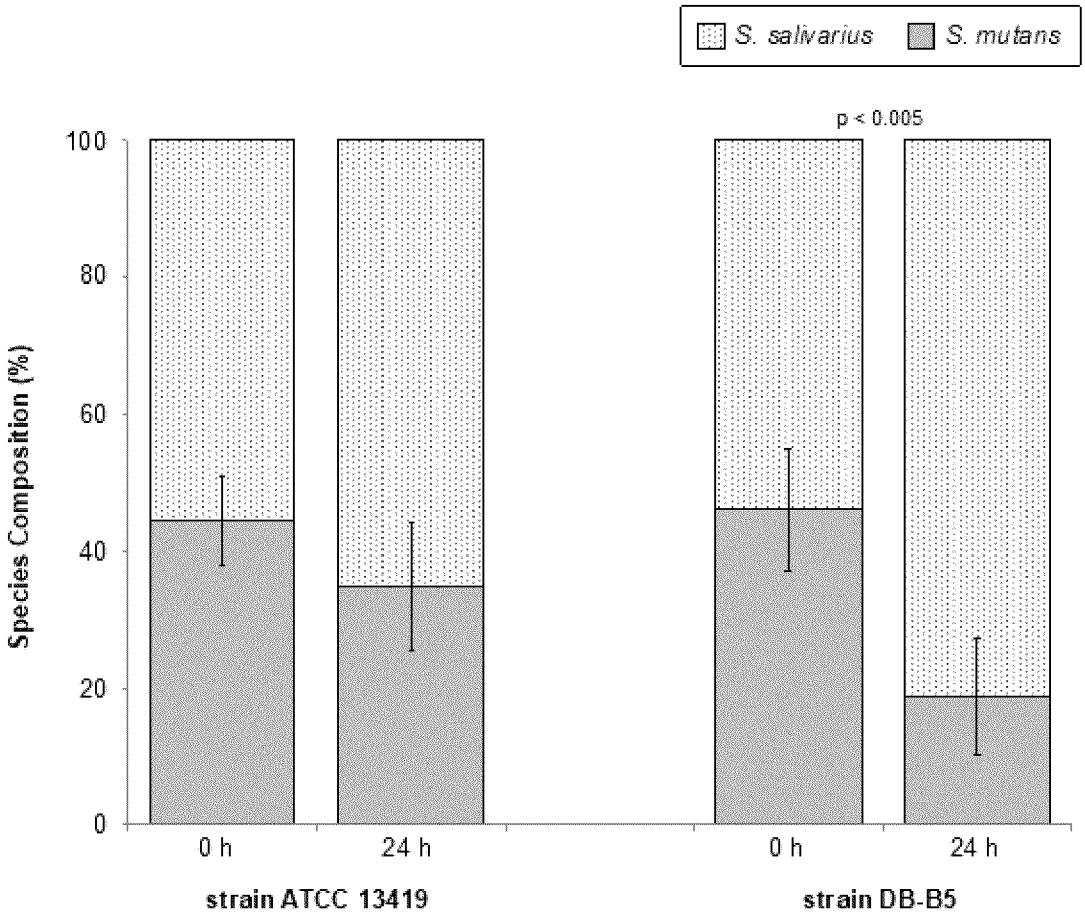
S. salivarius
S. mutans
p < 0.005
Species Composition (%)
100
80
60
40
20
0
0 h
24 h
0 h
24 h
strain ATCC 13419
strain DB-B5

FIGURE 2

A)

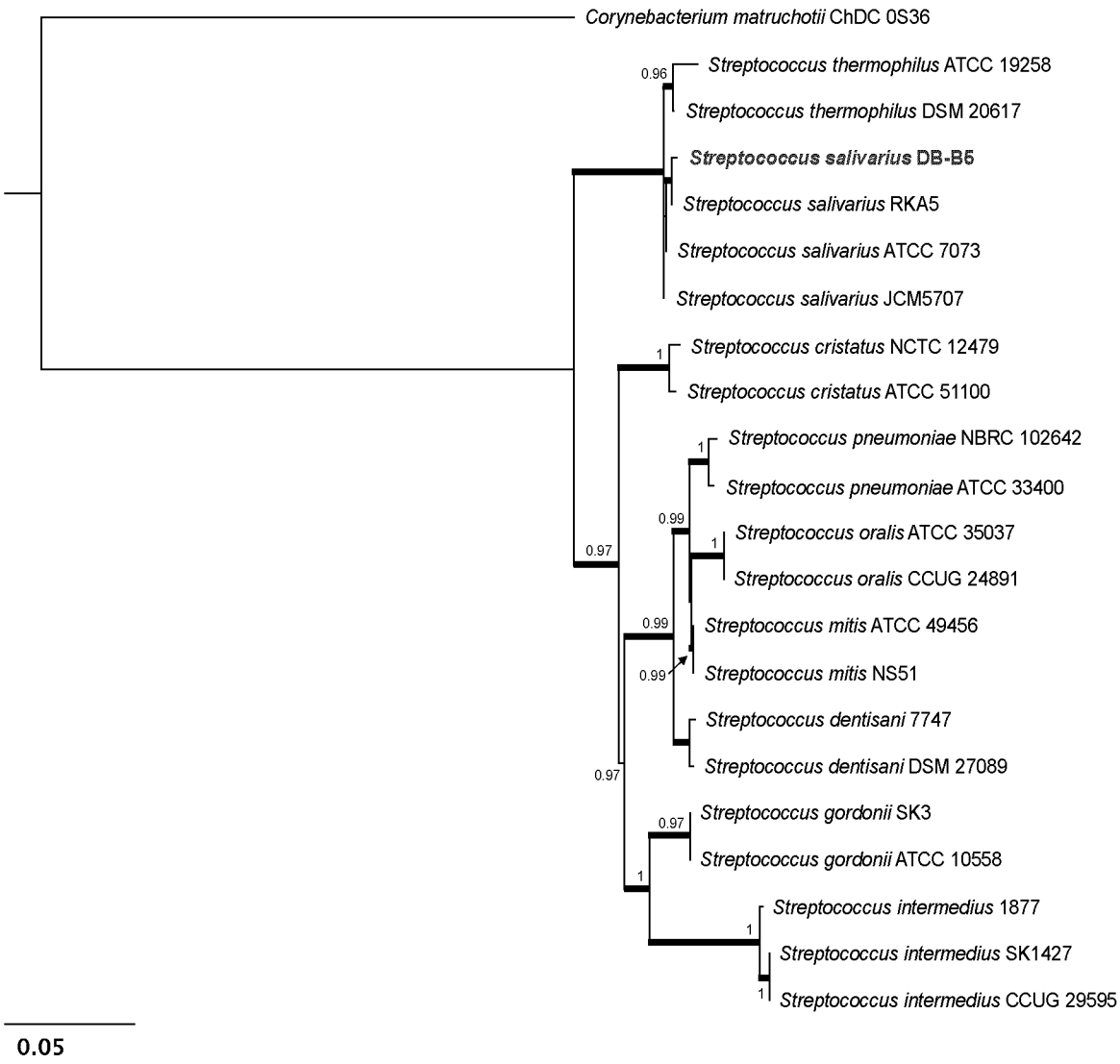
Corynebacterium matruchotii ChDC 0S36
0.96
Streptococcus thermophilus ATCC 19258
Streptococcus thermophilus DSM 20617
Streptococcus salivarius DB-B5
Streptococcus salivarius RKA5
Streptococcus salivarius ATCC 7073
Streptococcus salivarius JCM5707
1
Streptococcus cristatus NCTC 12479
Streptococcus cristatus ATCC 51100
1
Streptococcus pneumoniae NBRC 102642
Streptococcus pneumoniae ATCC 33400
0.99
1
Streptococcus oralis ATCC 35037
0.97
Streptococcus oralis CCUG 24891
0.99
Streptococcus mitis ATCC 49456
0.99
Streptococcus mitis NS51
Streptococcus dentisani 7747
Streptococcus dentisani DSM 27089
0.97
0.97
Streptococcus gordonii SK3
Streptococcus gordonii ATCC 10558
1
1
Streptococcus intermedius 1877
Streptococcus intermedius SK1427
1
Streptococcus intermedius CCUG 29595
0.05

FIGURE 2

B)

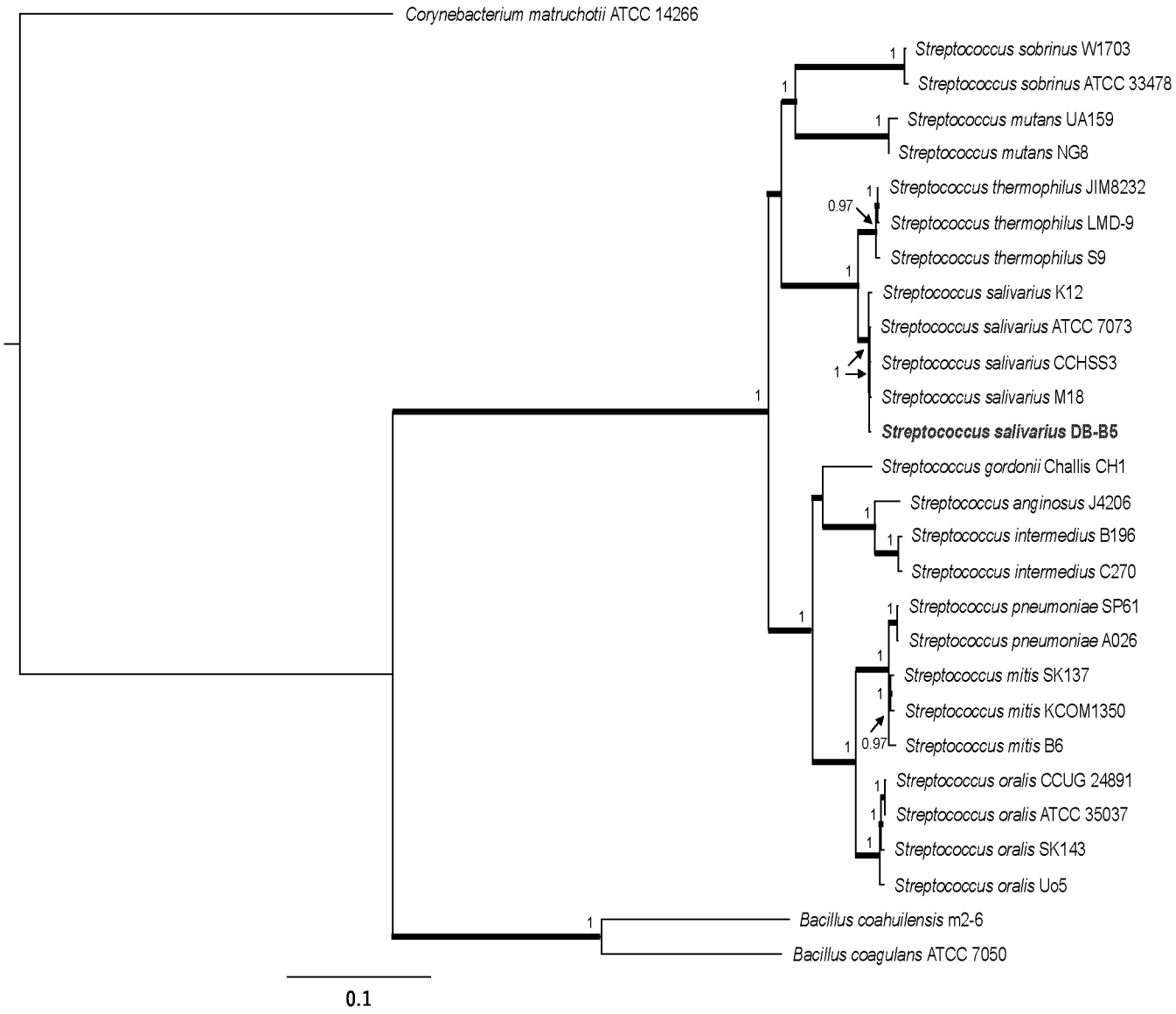
Corynebacterium matruchotii ATCC 14266
Streptococcus sobrinus W1703
Streptococcus sobrinus ATCC 33478
Streptococcus mutans UA159
Streptococcus mutans NG8
Streptococcus thermophilus JIM8232
Streptococcus thermophilus LMD-9
Streptococcus thermophilus S9
Streptococcus salivarius K12
Streptococcus salivarius ATCC 7073
Streptococcus salivarius CCHSS3
Streptococcus salivarius M18
Streptococcus salivarius DB-B5
Streptococcus gordonii Challis CH1
Streptococcus anginosus J4206
Streptococcus intermedius B196
Streptococcus intermedius C270
Streptococcus pneumoniae SP61
Streptococcus pneumoniae A026
Streptococcus mitis SK137
Streptococcus mitis KCOM1350
Streptococcus mitis B6
Streptococcus oralis CCUG 24891
Streptococcus oralis ATCC 35037
Streptococcus oralis SK143
Streptococcus oralis Uo5
Bacillus coahuilensis m2-6
Bacillus coagulans ATCC 7050
0.1

FIGURE 3

HRE60_06345 (hypothetical protein)
MMHKRNLIEDNKRGENQSFLYFLHEEKKFDVKALDDLCHYIIELDTISFEQLRDIHYIENQILRHLV
YHFDDNDLSKISNLPLEYWEYIEPFERLVACLYEGDGEE (SEQ ID NO:9)

HRE60_06350 (M13 family metallopeptidase)
MKKRKKIILGSITGALALALAGGGFWAYKTFVPQETPIDKNATVASNFYQAVNKDWLLKAKIPAD
SPSIDNFYTLGEDIKGKLKKDIKNLGEGKETSDITGMSEFITFYKAASDYKQREKDGLEPLKPYLKEI
EDIKDVNDLASKSASLTDKGIPLPFGYDVGTNAENTSQKQIQLSPPSILLPDVSIYKDEASKKQYLTP
IETATQKALEMLGYSEKNSKRIVKEALEFDEIIAKYSLSNEEMSESKNLVHPKTAEEINAYSGSFKL
YDVIKGIMGRDLETINVPNTKYFENYSKIVNQDNFSKIKSWILVQEAMAASNSLTEDYRLNFASISM
AIMGTQKPISKEDTVYEMSVNLFSDVMSVYYGRKYFGEEAKTDVTGMIDKIKNVYRGRLQQNDW
LTEDTRNKAIEKLDKMKVFVGYQEDVNPGTKELHLDPNKSFFELSEDIAQFGKRYTIDHFDDPIDK
NKWSGSAFDINAYYNPESNSINFPAGILQAPFYDKNQSVEKNYGGIGVVIGHEITHAFDSNGADYD
ENGDMHNWWTKADTKAFDKRIKAFEDQWNGLEIYGTKVNGKLTVTENVADAGGLSSTLQVLKT
DVTKPNLKDYFENYANIWKQKASLQYNKYTMVQDVHAPNELRVNQQLKNLPEFYEAYPQIKEGD
AMYLAPSKRISLW (SEQ ID NO:10)

HRE60_06355 (bacteriocin immunity protein)
MTKSTIIQIDDILNNIYNLIVNPETTENERELLVTYKNEIEVEKKDNDELLAELCRAIQALAVSNLSK
GKSLSSGVSDLSKTLTEFQEKSERNINLAKGLTSLGSLSFR (SEQ ID NO:11)

HRE60_06365 (helix-turn-helix domain-containing protein)
MLTKFGEIYKLFRESRKITLRDIEHRGISRSQLSRFEKGETDLTTTKFFLALEQINVPIEEFMYAVKDF
KRDCFYQLIDEVKQCFLNRNVPKLHKMLIKRMENDDNSIFSTIEIIFLKIKLQELSSEEYFNDTDINRI
TDYLFGVNYWGMFEILLFGNIMYIFNYETFLLLSKELLHRTEFYHDIPSYRRVIASIALNAFIICIERD
YLVDAKYFEKQISRFYFDESEIYERLIYTYARSFYEFKKEQTTQSILKMRKVIGFMRVVECEKLAER
YEEHLNKILSSFSDDN (SEQ ID NO:12)

HRE60_06370 (radical SAM protein)
MNERTWLEVFNRLYLTKDLSLNDAIKPIYVGYKIIERCNQRCVHCWAGKNDGVVPSLNDVISGLD
KLKQLQPLHLTITGGEPLMHEDWFSIVQYAKNNFPIVELFTNAVLFTEESVKRLSKIFDENDYVQVS
LDGLEKSYIKQRGVDDFKHVVTNIQLLVTYGINVRVNMTITHLNVNDMLGVYELLRDIGIASFAISP
VYPLRRGKKLIPLVDYDEYLVNSEKIKKLYSETKETMHLEIIYPIEIQSREARKLENSKDISRFNLDLL
HWTIDAKGDIFHFMDHFPIKELKIGNIYSNTTDELLSKDFKIQSSIMYHSSVGTKCSQCTLNDYCTGF
GYLNSYPEIASDFRRCSFEI (SEQ ID NO:13)

HRE60_06375 (radical SAM protein)
MKSENLIETCKKIESSFDLPFYIYSKISSKSNEKVIAPLKIGLKITNECHFRCPYCFVSKNSEYLRYQD
LKTVLSKLPQYPYEVYLTGGEATLHPKFDKIVDYIDSLGILIKLHTTGVVPLNTEKYILNNIEKFSSIQ
ISLDSIKNFNKLRPNLIDEDPLTQIVNFCKRLQQKKFENIIVNIVISSLNVMELPEIFDFCLSNKLKYIQ
LSSIFTTSKRLLTNDLDYLLYYEELLTTYQEQGIIFRTAPFCHPWSLAIQKGYDYRSPLYCPAQKTEF
EIDMHGDVYPCPFLHDEKHKMGNLLNNDFDEVWYSGVEELNHTNWSKNTKCKSCSLYKFCGGG
CYALASVSNKDYDMRCSLHAYK (SEQ ID NO:14)

HRE60_06380 (phosphoglycerate mutase)
MHINNKVWFIRFEPNFQGDKKTILYNKSSGELYEVSEIYFHFLESIRNKKSCQDALKQRYNTENIDIE
KIIQQIKGNLLNLGVILND (SEQ ID NO:15)

FIGURE 3 cont'd

HRE60_06385 (ABC transporter ATP-binding protein)
MTNITFSQVQKSFHKKLVLDIPDFQADSGEIFSIVGGNGAGKSTFIKLLAGIFLQDKGEVRVHGVSN
RSKKIHSLVKFVLESGQGLYSYLTAMENLQYFLGLNGIALSHIKAEVDVLCDQLAFTPYKDTLVSE
LSQGNRQKLTLILALVQKPKVLCLDEPTNGLDLLAKKQLMTLLQDYARYHQASIFITSHDASFIKK
VSTRVVVIQEGRLYRDGTFEEIFGNVHQHEVYHLLLDKSAESVLRQSFPELDYKVLDDGISVETRN
PDLYRLLLEETEVLQFTRESASLEDLLYEVLK (SEQ ID NO:16)

HRE60_06390 (ABC transporter)
MMLAAIGREFSRQLSEYKQFKVNLLFANLGIFFLVTGFLNYFDSQQDTFELFILLFTWYFSSHSITHP
TYFIEDEIADRTIINVIQSRRSIFGMLFIKIIVQILLDLVKGIPLFCLVTLVQQIAFPSDWVDTFVTFLLS
FVTIVSLYGLGFLFASFSFVFTKISSITSLLAYGILFLVGFEEQSSHLIATLSCFLPFHLLVSFIRQPSWF
VLLLLLGYGLFYWLLGYLCFQTCLTYAKKKGSLFHV (SEQ ID NO:17)

HRE60_06395 (hypothetical protein)
MYNEVKRFYLFRRRRWADTLFDTLYHVIFILGFYSLLRGNSDFQLSYFYYYFILTHVVSLGNEELE
YEIRSGQSFGIQYALQSVYRIYLQRAMVYFVWLSLIFWIALLLIHQGLPTHFSLEVINFQGLLVVLIG
FLIFLGYYLVMIRLTIRFQRISVLVDFLNTVLLFYSGLVFPVVSFGNLKKLFDGIIRN (SEQ ID NO: 18)

FIGURE 4

TCATTCCTCACCATCACCCTCATACAAACATGCCACCAAGCGTTCAAAGGGCTCTATATACTCCCAGTACTCTAAAGGGAGA
TTGGAAATCTTACTTAGATCATTATCATCAAAATGATACACAAGATGGCGTAGTATTTGATTTTCAATATAATGGATATCTCT
CAATTGCTCGAAACTGATTGTATCCAGTTCTATGATGTAGTGACACAAATCATCAAGTGCCTTTACGTCAAATTTTTTCTCCT
*CGTGTAGGAAGTAGAGAAAGCTCTGATTCTCACCTCTTTTATTATCTTCTATCAGATTCCTTTTATGCATCAT*TCTTGAATCCT
TTCCTTAATAGCACCATTATAACACTAGAAAATGATTCCCCAAAGCTACAACATAAAAAGAGAATCTGGGATGATTCCATTC
CAAATTCTCTTCTTCTTGATTAGCAAAGTAGCAGGACTTTT*TTACCACAAACTAATGCGCTTGCTTGGTGCTAAATACATGGC*
GTCTCCTTCCTTAATCTGAGGATAGGCCTCATAAAACTCAGGAAGGTTTTTAAGTTGTTGGTTAACACGCAACTCATTTGGT
GCGTGGACGTCTTGCACCATGGTATATTTATTGTACTGCAGGCTAGCCTTTTGTTTCCAGATATTTGCGTAGTTTTCAAAGTA
GTCCTTAAGATTAGGTTTTGTCACATCTGTTTTGAGCACTTGGAGTGTAGATGATAGACCTCCAGCATCTGCAACATTCTCA
GTAACTGTGAGTTTTCCATTTACCTTAGTCCCGTAAATTTCTAAACCATTCCACTGGTCTTCAAAGGCTTTGATTCTCTTGTCG
AAAGCTTTAGTATCTGCTTTTGTCCACCAGTTATGCATGTCGCCATTCTCATCATAATCGGCACCATTGGAGTCAAAGGCAT
GGGTAATTTCATGTCCAATCACGACACCGATACCACCATAGTTCTTTTCAACACTTTGGTTCTTGTCGTAAAACGGAGCTTGC
AAAATACCAGCTGGGAAGTTGATACTGTTACTTTCTGGGTTGTAATAAGCATTGATATCAAAAGCAGAACCAGACCATTTA
TTCTTATCAATAGGATCATCAAAATGATCAATGGTATAACGTTTTCCGAACTGGGCAATGTCTTCTGACAATTCAAAGAAGG
ACTTGTTAGGGTCAAGATGTAGTTCCTTAGTACCAGGATTGACATCTTCCTGATAACCTACGAAAACCTTCATTTTATCCAAC
TTTTCGATGGCCTTGTTGCGTGTATCTTCCGTCAACCAATCATTCTGTTGGAGACGGCCACGATAGACATTTTTGATTTTATC
AATCATACCTGTCACATCAGTCTTGGCTTCTTCACCAAAGTATTTACGTCCATAATAGACACTCATGACATCGCTAAACAGAT
TGACACTCATCTCATAGACTGTATCTTCTTTAGAAATTGGCTTTTGTGTGCCCATAATAGCCATGCTAATCGATGCAAAATTC
AAACGGTAATCTTCTGTCAAAGAATTTGACGCTGCCATAGCTTCTTGCACTAATATCCATGATTTTATTTTAGAAAAATTGTC
CTGATTAACGATCTTACTATAGTTCTCAAAATACTTGGTATTAGGGACGTTGATGGTTTCTAAATCACGTCCCATAATGCCTT
TAATGACATCATATAATTTGAAGGAACCCGAGTAGGCATTAATCTCTTCTGCTGTTTTAGGGTGAACCAAATTCTTACTTTCA
GACATCTCTTCGTTACTGAGACTGTACTTAGCAATGATTTCGTCGAACTCCAGTGCTTCTTTGACAATCCGTTTACTATTTTTT
TCGCTATATCCAAGCATTTCCAAGGCTTTTTGCGTGGCTGTTTCAATTGGAGTCAAATATTGTTTCTTGCTGGCCTCATCTTT
ATAAATAGACACATCTGGCAATAAGATACTTGGGGGACTCAATTGGATTTGTTTTTGGCTGGTATTCTCTGCGTTAGTTCCT
ACGTCATAACCAAATGGAAGAGGAATCCCTTTATCAGTCAAGCTAGCAGATTTACTGGCCAAATCATTTACATCCTTGATAT
CCTCAATCTCTTTTAGATAAGGTTTGAGAGGTTCAAGGCCATCTTTCTCACGTTGCTTGTAATCACTAGCAGCCTTGTAGAA
GGTGATAAACTCAGACATCCCCGTAATATCTGACGTCTCCTTACCCTCCCCTAAATTTTTGATATCCTTCTTAAGCTTTCCTTT
GATATCTTCCCCTAAAGTATAAAAATTATCAATGCTTGGAGAATCAGCGGGAATTTTGGCTTTTAGGAGCCAGTCCTTGTTA
ACAGCTTGATAAAAATTAGAAGCTACCGTAGCATTCTTATCAATTGGCGTTTCTTGCGGAACAAAGGTTTTATAAGCCCAAA
*AACCGCCTCCAGCAAGAGCTAAGGCCAAAGCTCCCGTTATGGAGCCAAGAATAATTTTCTTACGCTTTTTCAT*AAATACTCC
TCATAAAATTAAATTAATGTATTTGTATTATATCATTAGAACACTTATTTTAGCAAGCTGTTTTTGGGAGACAAAAAGAAGT
AATCAGACGATTACTTCTCTTTATTTTCTTAAATAGCGAAAATTAGGTATTATGACACTCAAGATAAGAACAATAAAAGATC
AACTAAATCCAACTTAGCAGGTTGAATTCAGTTAATCTTTTTGGTCATTTTACTAATCATCACAAGCAGATAAA*TCATCTAAA*
AGACAAACTGCCAAGTGATGTTAATCCTTTTGCTAGATTAATGTTACGCTCTGATTTTTCTTGGAATTCTGTGAGCGTCTTAC
TCAAGTCACTCACTCCAGAACTCAGTGATTTGCCTTTAGAAAGATTGCTGACTGCTAGAGCTTGAATAGCTCTACATAATTC
TGCAAGAAGTTCATCATTATCCTTCTTCTCAACTTCTATTTCATTTTTATACGTTACGAGCAACTCTCTTTCATTTTCAGTTGTT
*TCTGGATTTACAATCAGATTATAGATATTGTTTAAGATATCATCAATTTGGATAATAGTACTTTTTGTCAT*CTAGATTACCTC
CTATAACCTGTAGCAAGATTAGGTCGAGCAAGTGAATTAAATTGGTTAATTGTTTGTGTTGTCATGAGCAACGACCTCTTTT
AATAGTTCGATGTGACATCATTATACACATCTGGCATATCAAATTCAAGACTAAAAATATCCTTCTGCCACAAATGGTCCAA
ATTTAGCACCAACAGATAAAATAAAGTAATCAAACGATTACTTCTGTGATGCCCCCTTTTGTCAATTAAACCTTCTTCACCAT
CACTATCATACTTCTTTACCCACGAGTATACTTGGCTATAAGAAACATCATGAAGCACCGCCGTTTCCTTATAATTGCTATTG
TGTTCAATACAATAATTGACAATTTCTTTGCGCTCATCGATAGTAGTTTTACGTCTTGCTTTTGCCATATAGATCCCCCGTTTT
GGATTGTAATCCTTAAGTTCTATATTGGCAATATACATCCTAATCCACTTTCTTAAAAGTCCTGAAGAAATATCATACTGATG
ACCTAAATCAATAGATGAAGGCTCACTACTAATACATTTTTCTACAAGTTCCATTTTAAATTCTTTTGAATAGGAGCTGTTTT
TTGTTTTCTTTGTGAATCCTAAAATCCCATGTTTTTTTATAGATGGCAGCCCAATCTTGAATCGTCCTTGTGGAAATATGGAG
ACAGTTACATATTTCAGATCTGGAACGATTACCATTAATATAATCGAGACAAGCTTGTTCTTTCTCGTATGGGGTGAATCTT

FIGURE 4 cont'd

TCTCTTCTAGACATAAAAATACCTCTTAAGTAGATTTTAGGTATTTACCTTGTCTACTTAAGAGGTATCATATCATTAGACGG
GTGGTTTTGACTAATATAGGACCTAAATTAAAAAATGGTAATTATAAGGCGATGATAATGATAATTTCAAGTATAATCATT
AGGAATCTTTTTATAGAAATATTCATGTGAAACCTCCTTGAATAAATTAATAAAAACTTTAATTATATGATATAATCTATGTA
TATTTTATCACATGAAGAGACTTTATTTTAGAACTGTCCCATATTTGCTCATGGAGGTATAGAATGTTAACAAAATTTGGG
GAAATATATAAATTATTTAGAGAATCAAGAAAAATTACTCTTAGAGACATTGAGCACAGAGGGATATCACGTTCACAGC
TGTCTAGGTTTGAAAAAGGAGAAACAGATTTAACAACAACAAAATTTTTTTTAGCTCTTGAACAGATAAATGTACCCATC
GAGGAATTTATGTATGCTGTTAAGGATTTTAAAAGAGATTGTTTTTATCAACTAATAGATGAAGTCAAGCAGTGTTTTCT
TAATAGAAATGTGCCTAAATTACATAAAATGTTGATTAAACGCATGGAAAATGATGATAATTCTATCTTTTCCACAATAG
AAATTATTTTTTTAAAAATAAAATTACAAGAACTTTCTAGCGAAGAATATTTTAATGATACGGATATAAATAGAATAACT
GATTACTTATTTGGTGTTAATTATTGGGGAATGTTTGAAATTTTATTATTTGGAAATATCATGTACATTTTCAATTACGAA
ACGTTCCTATTGTTATCTAAAGAACTGTTACATAGAACAGAATTTTATCATGATATACCTAGTTATAGGAGAGTGATTGC
TAGTATAGCTCTAAATGCTTTTATTATCTGTATTGAAAGGGATTACCTAGTGGATGCAAAATATTTTGAAAAACAAATTT
CTCGTTTTTATTTTGATGAATCTGAGATTTATGAGCGGTTAATTTATACATACGCACGTTCTTTTTATGAATTTAAAAAAG
AGCAGACAACACAATCAATCCTAAAAATGAGAAAAGTAATTGGATTTATGCGTGTAGTCGAATGTGAGAAACTTGCAG
AGAGATATGAAGAACATTTAAATAAAATATTGTCTTCCTTCTCAGATGATAATTAAAAGAGCAGATTCGGGACAAAATA
GAATTAGATTTATAAAGTAATATACTATACTCGTAAGCTTACAAATACAATTTGAAGTAAGGGAGGGATAGTCATGTCTAG
TTTAGAGAAACATGAACTGATGGAAACTAAACTAACAGCAATCGATTTGTCGGTAGAAAAAATTGATTGCGGAAAGATTA
AGTTATACAAATCATTGTCTAAATCACATGGTCATTAATGAGCTTTATATTGATTAGCGATGATATTTGTGAAGTAAAATGA
GAGAATTTTAATTTCCTGTATGATTATTGGTGTGGTTAAAATTCTCTCATTTTTTATCAAATTAAGAAACTTGGTGCATTTTA
TGAATGAAAGAACTTGGTTGGAAGTTTTTAATCGTTTGTATTTAACAAAAGATTTATCGTTAAATGATGCAATAAAGCCT
ATCTATGTAGGTTATAAAATTATTGAGAGATGTAATCAACGTTGTGTCCACTGCTGGGCTGGAAAAAATGATGGGGTG
GTACCGTCATTAAATGACGTTATATCAGGACTTGATAAACTTAAACAACTTCAACCTCTTCATTTAACCATAACAGGTGG
AGAACCTTTAATGCATGAAGATTGGTTTTCAATAGTTCAATATGCAAAAAACAATTTTCCAATTGTTGAGTTATTCACTA
ATGCTGTTTTATTTACAGAAGAATCTGTGAAGAGATTATCAAAAATTTTTGATGAAAATGACTATGTTCAAGTGAGTTTG
GACGGTTTAGAAAAGAGTTACATAAAACAGAGAGGTGTAGATGATTTCAAGCATGTGGTGACAAATATTCAATTACTA
GTGACTTATGGCATTAATGTTCGAGTTAATATGACTATTACTCATCTAAATGTTAATGATATGTTAGGAGTATATGAGTT
GTTAAGAGATATTGGAATAGCTTCGTTTGCTATCTCTCCAGTTTATCCGTTAAGAAGAGGAAAGAAATTAATTCCGTTAG
TTGATTACGATGAATACCTTGTAAATTCTGAAAAGATAAAAAAACTGTATTCTGAAACTAAAGAAACAATGCATCTTGA
AATAATTTATCCAATAGAAATTCAATCTAGAGAAGCTAGAAAATTGGAGAATTCGAAAGATATTTCTCGTTTCAATTTAG
ATTTATTACATTGGACAATAGACGCAAAGGGAGATATATTTCATTTTATGGATCATTTTCCTATAAAAGAACTCAAGATA
GGGAATATATATTCTAACACTACTGACGAGTTACTATCAAAGGATTTTAAGATTCAATCCTCTATTATGTATCATAGTTCG
GTGGGAACAAAATGTTCACAGTGTACCTTGAATGATTATTGTACGGGATTTGGATATCTTAATTCCTACCCTGAGATTGC
TTCAGATTTTAGGAGGTGTAGCTTTGAAATCTGAAAATTTAATCGAAACATGTAAAAAAATAGAGTCAAGTTTTGACTT
GCCATTTTATATCTATTCAAAGATAAGCTCTAAAAGCAATGAAAAAGTAATTGCGCCTTTGAAGATTGGTCTGAAAATA
ACTAATGAATGTCATTTTAGATGTCCTTATTGTTTTGTAAGTAAGAATTCGGAATATTTGAGATATCAGGATTTGAAAAC
TGTTTTGTCCAAATTACCTCAATATCCATACGAAGTTTATTTGACTGGGGGAGAAGCAACTCTTCATCCGAAATTTGATA
AAATTGTTGATTATATAGATAGTTTAGGTATTTTGATTAAATTACATACGACGGGTGTAGTTCCCCTTAATACAGAAAAA
TATATATTAAATAATATTGAAAAATTTTCATCGATTCAAATTAGTTTAGACTCAATAAAGAATTTTAATAAATTGAGACC
AAATCTAATTGATGAAGATCCTTTGACTCAAATTGTTAATTTTTGTAAAAGATTACAACAAAAGAAATTTGAAAATATTA
TAGTTAATATTGTAATAAGCTCTCTAAATGTAATGGAGCTTCCAGAAATTTTTGATTTTTGTTTATCTAATAAGTTGAAAT
ATATTCAATTATCATCAATATTTACTACGAGTAAACGATTGTTAACTAATGATCTTGACTACTTGCTTTATTATGAGGAAT
TATTAACTACTTATCAAGAACAAGGAATAATTTTTAGAACAGCTCCTTTTTGTCATCCATGGTCTTTGGCTATTCAAAAAG
GTTATGATTACCGATCCCCTTTATATTGTCCAGCACAAAAAACAGAGTTTGAGATAGATATGCATGGCGATGTTTATCCT
TGCCCGTTTTTGCACGATGAAAAACATAAAATGGGGAATTTACTGAATAATGACTTTGATGAAGTATGGTATTCTGGAG
TAGAAGAACTAAACCATACAAATTGGAGCAAAAATACTAAATGTAAATCCTGTTCACTATATAAATTTTGTGGCGGAGG
TTGTTATGCTCTGGCGAGTGTGTCTAATAAGGATTATGATATGAGGTGTAGTTTACATGCATATAAATAATAAAGTGTG
GTTTATTCGTTTTGAGCCTAATTTTCAAGGAGATAAAAAGACAATACTATATAATAAGTCTAGCGGCGAACTATATGAA
GTATCTGAAATATATTTTCATTTTTTAGAAAGTATAAGAAATAAGAAAAGTTGTCAAGATGCTTTAAAGCAACGATATA

FIGURE 4 cont'd

ATACTGAAAATATAGATATTGAAAAAATAATTCAACAAATTAAAGGGAATCTTCTAAATCTAGGGGTTATCTTAAATGA
CTAACATAACATTTTCACAAGTTCAAAAGAGTTTTCACAAGAAGTTGGTGTTAGACATTCCTGATTTTCAAGCGGATTCA
GGTGAAATCTTCTCCATCGTTGGTGGCAATGGTGCCGGTAAGTCGACTTTTATCAAGCTCTTGGCAGGGATTTTTTTGCA
GGATAAGGGCGAGGTTCGTGTTCATGGCGTTTCTAATCGCTCCAAGAAAATCCACTCGCTGGTCAAGTTTGTCTTGGAG
AGCGGGCAGGGGTTGTACAGTTATTTGACAGCTATGGAAAATCTTCAATATTTTCTAGGATTGAATGGGATTGCCTTGT
CTCATATCAAGGCAGAGGTGGATGTCTTGTGTGACCAGCTGGCTTTTACCCCTTATAAGGACACGCTGGTTTCAGAACTG
TCCCAGGGCAACCGTCAGAAATTGACCTTAATCTTAGCTTTAGTACAGAAACCGAAAGTACTCTGTTTGGATGAGCCGA
CTAATGGGCTAGATTTGCTGGCAAAAAAACAGCTGATGACTCTTTTACAGGATTATGCTCGTTACCATCAAGCAAGTATT
TTCATCACCAGTCATGACGCTAGTTTTATCAAGAAGGTCAGCACTCGGGTGGTGGTAATTCAGGAGGGGCGGCTTTATC
GTGACGGAACCTTTGAAGAGATTTTTGGCAATGTCCACCAGCATGAAGTCTATCACTTGCTTCTAGATAAGAGTGCAGA
AAGTGTACTGAGACAAAGCTTTCCTGAGCTGGACTACAAGGTGCTTGATGATGGGATTTCGGTAGAGACCAGAAATCC
AGACCTTTATCGGCTATTGCTAGAAGAAACGGAAGTCTTGCAGTTCACCCGAGAATCTGCCTCTTTGGAAGATTTGCTGT
ATGAGGTGCTCAAATGATGCTAGCAGCGATTGGGCGAGAGTTTAGTCGCCAACTCAGTGAATACAAACAGTTCAAAGT
CAATCTTCTCTTTGCCAATCTGGGAATTTTCTTTCTGGTTACGGGTTTTCTGAACTATTTTGACAGTCAGCAAGATACTTTT
GAGCTGTTTATATTGCTTTTTACTTGGTATTTTTCTAGCCACAGTATCACTCATCCGACCTATTTTATCGAAGATGAGATTG
CAGACCGTACCATTATCAACGTGATTCAGAGTCGGCGGAGTATCTTTGGCATGCTTTTTATCAAAATTATCGTACAGATT
TTGTTGGACTTGGTCAAGGGCATTCCTTTGTTTTGCTTAGTTACCTTGGTTCAGCAGATAGCCTTTCCTAGTGACTGGGTC
GATACTTTTGTTACCTTTCTTCTGTCCTTTGTGACCATTGTATCCCTTTATGGTCTGGGCTTTCTCTTTGCCAGTTTTTCCTTT
GTCTTTACAAAAATTTCCAGTATTACTAGCCTCCTTGCTTACGGGATTCTCTTTTTGGTTGGTTTTGAGGAGCAATCGAGT
CATTTGATTGCTACTTTATCTTGTTTCTTGCCTTTTCATCTCTTGGTGAGTTTTATCAGACAGCCAAGTTGGTTCGTTCTTTT
ATTATTACTGGGCTACGGTTTGTTTTACTGGCTCTTGGGCTACTTGTGCTTTCAAACTTGTTTGACCTATGCCAAAAAGAA
GGGAAGTTTGTTCCATGTATAATGAAGTGAAGCGATTTTATCTCTTTCGCAGGAGACGCTGGGCGGATACCCTGTTTGA
CACCCTCTACCATGTCATTTTCATTCTTGGATTTTATAGCCTTTTGAGGGGAAATTCTGACTTTCAGTTGTCCTATTTCTATT
ATTATTTCATCTTGACCCATGTGGTTTCCTTGGGCAATGAAGAGTTGGAGTACGAGATTCGGTCGGGGCAATCTTTTGGC
ATTCAGTATGCTTTGCAATCAGTGTATAGAATTTACCTTCAACGAGCTATGGTTTACTTTGTTTGGTTGTCTTTGATTTTCT
GGATTGCTCTGCTTTTGATTCATCAGGGTTTACCAACTCACTTTTCTTTGGAGGTCATCAATTTCCAAGGACTGCTCGTGG
TATTGATTGGTTTCTTGATTTTTCTCGGCTATTATCTAGTCATGATTCGACTGACCATTCGCTTCCAACGGATTTCAGTCTT
GGTAGATTTTCTAAATACCGTGCTTTTATTCTACTCAGGACTCGTCTTTCCAGTGGTGAGTTTTGGGAATTTGAAGAAATT
ATTTGACGGGATTATAAGAAATTAA (SEQ ID NO:19)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2021/051001

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 1/20*** (2006.01) , ***A23L 33/135*** (2016.01) , ***A61K 35/74*** (2015.01) , ***A61K 35/741*** (2015.01) , ***C07K 14/315*** (2006.01)

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)
C12N 1/20 (2006.01) , ***A23L 33/135*** (2016.01) , ***A61K 35/74*** (2015.01) , ***A61K 35/741*** (2015.01) , ***C07K 14/315*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel-Orbit, Genomequest, Scopus, Google Scholar
Keywords:Streptococcus, salivarius, DB-B5, subtilosin, bacteriocin*, oral pathogen*, protease, resistant, lantibiotic, sactipeptide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BURTON, J.P., et al., "Persistence of the oral probiotic *Streptococcus salivarius* M18 is dose dependent and megaplasmid transfer can augment their bacteriocin production and adhesion characteristics", PLoS ONE, Vol 8, No. 6, e65991, 13 June 2013 (13-06-2013), ISSN: 1932-6203 *page 2, Table 1*	1-5, 8-21, 23-25
A	DI PIERRO, F., et al., "Preliminary pediatric clinical evaluation of the oral probiotic *Streptococcus salivarius* K12 in preventing recurrent pharyngitis and/or tonsillitis caused by *Streptococcus pyogenes* and recurrent acute otitis media", Int. J. Gen. Med., Vol. 5, Pages 991-997, 29 November 2012 (29-11-2012), ISSN: 1178-7074 *abstract, page 992, Tables 2 and 3*	1-5, 8-21, 23-25

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"D"	document cited by the applicant in the international application	"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
"E"	earlier application or patent but published on or after the international filing date	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 21 September 2021 (21-09-2021)	Date of mailing of the international search report 04 October 2021 (04-10-2021)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Debora Fujimoto (873) 335-9738

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WESCOMBE, P.A., et al., "Megaplasmids encode differing combinations of lantibiotics in *Streptococcus salivarius*", Antonie Van Leeuwenhoek, Vol. 90, No. 3, Pages 269-280, e-published 27 July 2006 (27-07-2006), ISSN: 0003-6072 *whole document*	1-5, 8-21, 23-25
A	HOLS, P., et al., "Mobilization of microbiota commensals and their bacteriocins for therapeutics", Trends Microbiol., Vol. 27, No. 8, Pages 690-702, 2019, ISSN: 0966-842X *Pages 691 and 693-698, Table 1*	1-5, 8-21, 23-25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2021/051001

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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: