Available online at www.sciencerepository.org

Science Repository

Review Article

Review of Oral Probiotics and the Methods Useful in Study of Dental Biofilms and for Selection of Potential Beneficial Bacteria and Their Products for Development of Oral Probiotic

Maďar Marián[1*], Fedorová Monika[2], Stašková Andrea[1,3], Styková Eva[4], Jana Kačírová[1], Lauko Stanislav[1], Englerová Karolína[1], Gabriela Štrkolcová[5], Soňa Gancarčíková[1] and Nemcová Radomíra[1]

[1]*Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Kosice, Komenskeho 73, Košice, Slovakia*
[2]*Department of Pharmacy and Social Pharmacy, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, Košice, Slovakia*
[3]*Clinic of stomatology and maxillofacial surgery, Faculty of Medicine, University of Pavol Jozef Safárik in Košice, Akadémia Košice, n.o., Bačíkova 7, Košice 040 01, Košice, Slovakia*
[4]*Clinic for horses, University of Veterinary Medicine and Pharmacy in Kosice, Komenského 73, Košice Slovakia*
[5]*Department of Epizootiology and Parasitology, University of Veterinary Medicine and Pharmacy in Kosice, Komenskeho 73, Košice, Slovakia*

ARTICLE INFO

Article history:
Received: 7 January, 2019
Accepted: 21 January, 2019
Published: 31 January, 2019

Keywords:
Potential oral beneficial bacteria
methods
streptococcus
lactobacillus

ABSTRACT

Colonization of the oral cavity begins immediately after birth, however, only some micro-organisms are capable of exerting their action in the oral environment. A wide range of micro-organisms is found in the oral cavity, whether commensal, facultative pathogenic or obligatory pathogenic. Their mutual ratios and numbers are considerably affected by probiotic bacteria of the oral microbiota, particularly by their products, such as bacteriocins. The probiotics most frequently living in the oral cavity include Lactobacillus reuteri, Streptococcus salivarius and Bacillus coagulans. This study is focused on oral probiotics which could improve the health of the oral cavity and prevent development of dental carries. Basic techniques, which are necessary in research of oral microbiota that could be potentially beneficial in dental microbiology, are also presented. We recommended these techniques based on our experiences in this field. In this study we describe microbiological methods for obtaining of live bacterial strains in dental plaque or dental calculus, that are used for preparation of the bacterial strains´ collection and their storage possibilities for next testing. Study also describes two sensitive molecular methods usable for identification of these bacterial strains, the first with help of 16S rRNA and next blast n analysis based on consensus DNA sequences, and the second based on MALDI-TOF mass spectrometry. PCR methods for deeper characterization of selected bacterial strains e.g. pathogenic like Streptococcus mutans or potentially beneficial bacteria such as Str. salivarius and Lactobacillus spp. are also described. These methods are based on detection of genes coding production of bacteriocins or coding genes responsible for pathogenicity e.g. glucosyl or fructosyl transferaze genes. We also recommend the method for detection of hard cultivable spirochetes based on the morphological characteristics with help of VFQTOPF method and next by PCR methods used for detection of Treponema denticola in dental plaque samples.

*Correspondence to: *Maďar Marián, Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Kosice, Komenskeho 73, Košice, Slovakia; E-mail: madarmarian@gmail.com*

http://dx.doi.org/10.31487/j.DOBCR.2019.01.001

Introduction

It has been well known that colonization of the oral cavity starts immediately after birth of an individual and continues throughout lifetime [1]. Oral cavity is an open system; many bacterial species are introduced to this environment with an uninterrupted introduction and removal of nutrients. It offers diverse habitats where-in different species of micro-organisms can prosper. However, only some of them are capable of surviving and even thriving in this seemingly favorable environment [2]. They must adhere to a suitable surface, colonize it and then, in collaboration with other bacteria, produce biofilm important for their maintenance in the oral cavity [3]. Some of the bacteria that enter the mouth will remain only transiently as they are not adapted to thrive in this environment. It is currently estimated that around 1,000 species of bacteria are able to exist stably in the human mouth, and that individuals typically maintain between 50–200 species from this wider pool [4].

Oral microbiota

Bacteria colonize the teeth, tongue, oral mucosa, hard palate, carious lesions, periodontal pocket and similar. Microbiota of the oral cavity is not distributed at randomly, most species show affinity to certain sites and prefer them to others with regard to specific local conditions provided by these sites, such as the anaerobic environment in the periodontal pocket [5]. The easily cultivable bacteria found in the oral cavity belong to the following genera: *Streptococcus, Actinomyces, Veillonella, Fusobacterium, Porphromonas, Prevotella, Treponema, Neisseria, Haemophilis, Eubacteria, Lactocillus, Capnocytophaga, Eikenella, Leptotrichia, Peptostreptococcus, Staphylococcus*, and *Propionibacterium* [6, 7]. However, there are also bacteria that cannot be detected by conventional cultivation methods, therefore, molecular methods have to be used. Proportion of these bacteria can be as high as 60 % [8].

Differences in composition of the oral microbiota are considerably influenced by external factors, such as food, drink, living temperature and humidity [9]. Moreover, smoking, saliva flow, general health, etc, can lead to overgrowth by previously minor components of the oral microbiocenosis [10]. The oral microbiocenosis of the elderly is different from that of younger individuals. Age-related factors that can influence the composition of the oral microbiocenosis are dentures, hormones, long-term medication and reduced oral hygiene [11].

Diseases of the oral cavity and pathogenicity of micro-organisms

Dental caries is one of the oldest and most common diseases found in humans [12]. Despite all the achievements of dental medicine, it affects more than 80% of the world's population [13]. *Streptococcus mutans* has been implicated as the major causative agent of dental caries [14]. Now it is known that dental caries lesions contain a range of streptococcal species and members of the genera *Actinomyces*, *Bifidobacterium, Lactobacillus, Propionibacterium, Veillonella, Selenomonas, Atopobium* [6, 15]. Pathogenic microbiota also play an important role in periodontal diseases known as gingivitis and periodontitis [16]. Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Micromonas micros, Campylobacter rectus and Prevotella melaninogenica are considered as most common pathogenic bacteria caused inflammatory disease [17, 18]. Oral micro-organisms can also act as opportunistic pathogens and cause serious diseases in other body compartments [19]. These bacteria can affect the course and pathogenesis of several systemic diseases, such as cardiovascular disease, bacterial pneumonia, diabetes mellitus, and low birth weight [20]. It was shown that administration of probiotic tablets harmonized oral microbiota in patients suffering from periodontitis or gingival inflammation in comparison with the control group [21].

Oral probiotics

Probiotics have a beneficial effect on digestive system, but also potential favourable effect of probiotics on the vaginal mucosa, urinary tract and skin [22-24]. Key features of an effective probiotic for use in substitution therapy include: the absence of virulence determinants, the ability to colonize, and the ability to competitive suppress of target bacterium [25]. In the oral cavity, probiotics hinder the formation of dental plaque, the biofilm build-up on teeth, by blocking the attachment of microorganism to the surface of teeth [26]. Furthermore, they compete with the bacteria of the oral cavity for nutritive sources, produce chemical substances that lead to the inhibition of the development of pathogenic bacteria, facilitate and adjust the local specific and unspecific immune response, as well as provide other non-immunologic defense mechanisms [26, 27]. Probiotics poses a great potential in area of halting, altering, or delaying periodontal diseases. They play crucial role in terms of plaque modification, halitosis management, altering anerobic bacteria colonization, improvement of pocket depth, and clinical attachment loss [28]. Oral probiotics are living bacteria that are similar or identical to beneficial bacteria found naturally in the oral cavity [29]. In respect to commensal oral microbes, several aspects support the idea that it may be possible to find bacteria that could be useful in prevention or treatment of oral diseases [30]. Strains belonging to the *Lactobacillus*, *Streptococcus* and *Bifidobacterium* genera are most frequently used probiotics in the oral cavity [29, 31]. The main focus in the studies of potential oral probiotics is on caries prevention, especially on the possibility of reducing the number of mutans streptococci or their acidogenic activities in dental plaque when products containing certain probiotic strains are used [32, 33]. In recent years, there is an increasing interest in a new method of caries control, which depends on the ability of certain bacteria to produce BLIS [33, 34]. Probiotics also could have an important role to play in the clinical management of the periodontal diseases, although the evidence is less convincing as regards halitosis [35].

Lactobacilli as oral probiotics

Lactobacilli isolated from the oral cavity, were tested and their favourable effects on oral health were observed [32]. These bacteria produce different antimicrobial compounds such as organic acids (primarily lactic and acetic acid), hydrogen peroxide, and antimicrobial peptides, including bacteriocins [36]. The species of L. acidophilus, L. crispatus, L. delbrueckii, L. gasseri, L. salivarius, L. paracasei, L. plantarum, L. rhamnosus, L. fermentum and L. oris were recovered from the oral cavity of humans and it was proved, that they exerted inhibitory action on periodontal pathogens in in vitro conditions [37]. They have an antagonistic effect also on Streptococcus mutans, one of the major cariogenic organisms. Probiotic species of lactobacilli found in

commercial products (L. rhamnosus, L. casei, L. reuteri a Bifidobacterium lactis) adhered to hydroxyapatite coated by a layer of saliva and reduced adhesion of S. mutans under in vitro conditions [32]. The consumption of yogurt containing *L. reuteri* for 2 weeks showed significant growth inhibitory effect against *S. mutans* [38]. Several next studies investigating effects of oral probiotics showed that consumption of products containing probiotic lactobacilli decreased the risk of development of caries and the counts of S. mutans in the oral cavity. The consumption cheese containing *L. casei LAF-TI-L26* (1×10^6 Cfu /g) twice daily for two weeks leads to statistically significant ($p = 0.001$) reduction of salivary Streptococcus mutans [39]. It was observed that administration of Lactobacillus salivarius TI2711 (LS1) neutralized salivary pH in healthy individuals. Peroral administration of tablets containing L. salivarius WB21 improved periodontal health in healthy volunteers, particularly in smokers [40, 41]. Bacterial quantitative analysis found significantly lower levels of ubiquitous bacteria and Fusobacterium nucleatum during administration of L. salivarius WB21 [42]. The prevalence of DNA of the species L. salivarius in the oral cavity in the group with physiological halitosis was higher compared to that in the group with pathological halitosis [43]. L. salivarius BGHO1, isolated from a healthy oral cavity inhibited growth of other bacteria including S. mutans [44]. On the contrary, in vivo experiments on rats showed that a combination of L. salivarius LR1952R and S. mutans MT8148 considerably increased the incidence of dental caries in comparison with only S. mutans MT8148 [45]. Other studies did not report the adverse effects of administration of L. salivarius WB21 for eight weeks, and patients who continued taking L. salivarius WB21 for 3 months showed no new caries [40]. However, Lactobacillus salivarius WB21 administered in the form of tablets failed to reduce the incidence of any periodontopathic agent (Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola and Aggregatibacter actinomycetemcomitans) (Mayanagi et al., 2009). The findings concerning probiotic lactobacilli and cariogenicity differ. Considerable intra- and inter-individual differences were observed in the occurrence of individual Lactobacillus spp. and lactobacilli counts but no lactobacilli species were associated with plaque acidogenicity [46]. Some lactobacilli species were isolated in high counts from surface and deep dental caries [47], however, lactobacilli were isolated also from healthy oral cavity [48, 49]. Higher prevalence of L. gasseri and L. ultunensis was detected on dentine containing caries lesions [48]. Examinations involving periodontology showed the prevalence of L. gasseri and L. fermentum in healthy individuals while L. plantarum prevailed in patients with chronic periodontitis [49]. Lactobacillus reuteri can be used to reduce gingival inflammation and dentine plaque in patients with medium to serious gingivitis and can also decrease proinflammatory cytokines in gingival fluid (Krasse et al., 2006; Twetman et al., 2009). From the healthy oral cavity were mostly isoltated L. fermentum, L. plantarum, L. salivarius and L. rhamnosus [48].

Streptococcus salivarius as oral probiotics

Streptococcus salivarius is Gram-positive bacterial comensus and a progressive oral cavity coloniser, producing ribosomally synthesized protein bacteriocins. Some strains of Streptococcus salivarius such as K12 and M18, are currently used as probiotics worldwide due to their ability to produce various types of bacteriocins, called lantibiotics such as salivaricin A, salivaricin B, salivaricin 9, and salivaricin G32 [50]. They are safe for the consumer, do not induce inflammation and do not damage the non-pathogenic microflora of the oral cavity. These two strains specifically reduce the levels of cariogenic pathogens in the mouth, also by production of bacteriocin like inhibitory substances (BLIS) [51]. Generally, these BLIS proteins prevent from many oral health disorders, such as halitosis or dry mouth, particularly by suppressing growth of other micro-organisms present in the mouth. For example, BLIS produced by bacterium Streptococcus salivarius K12 was named BLIS K12 [52]. BLIS K12 is also name of commercially available product now available in chewable tablets, fast-soluble tablets, lozenges, chewing gums and powders [51]. Toxicology studies in animals did not reveal any adverse effect of multiple dosing Streptococcus salivarius K12. Meanwhile, Streptococcus salivarius K12 assessed for tolerance and safety in humans in a randomized, placebo-controlled trial [51]. Streptococcus salivarius is a non-pathogenic predominant colonizer in the oral microbiome, reduces the frequency of colonization of major pathogens involved in upper respiratory tract infection [53]. Streptococcus salivarius strain K12, are often used as probiotic bacteria in the treatment of streptococcal pharyngitis, tonzilitis and acute otitis media [54]. The strains Streptococcus salivarius K12, Streptococcus salivarius (RS1) and (ST3) bind effectively to FaDu human epithelial cells of the pharynx and in this way antagonise Streptococcus pyogenes its adhesion and growth. However, the strains RS1 and ST3 do not possess genes encoding production of bacteriocins, while the strain K12 strain provides a positive signal for two bacteriocins, lantibiotics: Salivaricins A and B from Streptococcus salivarius (ST3) and (RS1) inhibit the growth of Streptococcus pyogenes [55]. A recent study showed that the oral probiotic Streptococcus salivarius K12 can induce anti-inflammatory response in epithelial cells under in in vitro conditions, which indicates potential support of cell health and homeostasis [56]. Streptococcus salivarius, found in oral probiotics, naturally destroys Streptococcus mutans and thus prevent susceptibility of teeth to dental caries [32]. Streptococcus salivarius produces urease and dextranase which helps to dissolve and release the sticky dextran. Activity of these exoenzymes helps to reduce the progression of dental caries by reducing accumulation and acidification of dental plaque created by Streptococcus mutans [25]. Streptococcus salivarius K12 has shown a therapeutic potential in the treatment of halitosis [52]. Halitosis is caused by volatile sulphur compounds produced by bacteria present on the tongue, teeth, throat and tonsils. It has been shown that Streptococcus salivarius K12 is active against bacterial species involved in halitosis, by inhibition of Micrococcus luteus Il, Streptococcus anginosis T29, Eubacterium saburreum ATCC 33271 a Micromonas micros ATCC 33270 [57]. Twenty-three halitosis subjects underwent a 3-day application of chlorhexidine, followed by the administration of lozenges comprising Streptococcus salivarius K12. The assessment of levels of volatile sulfur compounds in 85% of the subjects showed a significant reduction (> 100 ppb) [58]. Streptococcus salivarius K12 also into In vitro conditions demonstrated their ability to modulate Candida albicans growth, which is causer of oral candidosis [59]. Interestingly, Streptococcus salivarius K12 was not directly fungicidal but inhibited the adhesion of Candida albicans to a plastic Petri dish. Candida albicans cultivated in the presence of Streptococcus salivarius K12 retained the morphological shape and size of the cells, compared to the group without Streptococcus salivarius K12, however, the adhesion of the mycelial form was weaker and the number of mycelium was reduced by the dose of lyophilized starting material Streptococcus salivarius K12 at a concentration of more than 0.94 mg / ml [59].

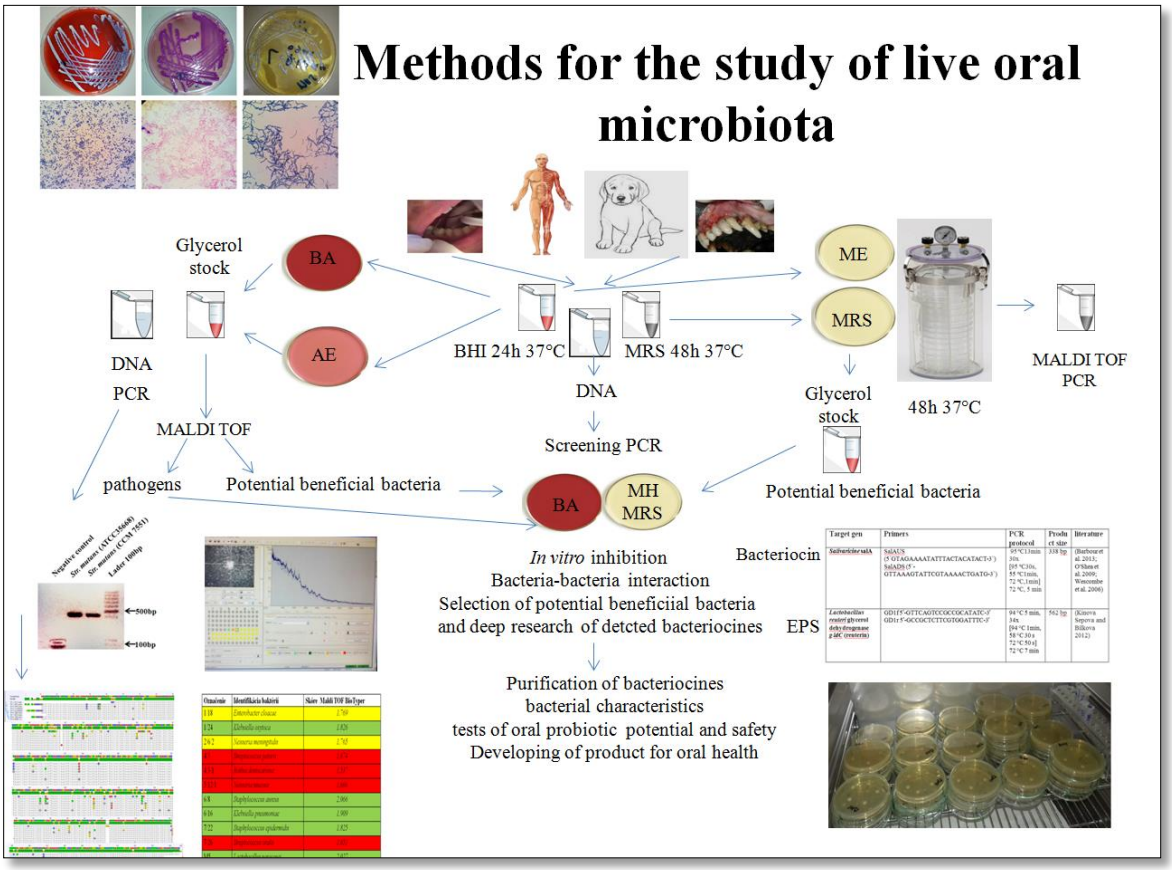

Figure 1: Schematic description of obtaining of sample and classical cultivation method used in research of potential beneficial bacteria Lactobacillus reuteri or other lactic acid bacteria and Streptococcus salivarius from dental plaque.

Material and Methods

This part of research presents the methods which we recommend to other researchers for the study of oral microbiome based on self-experiences. Methods descriptions see in (Figure 1).

Taking samples:

Obtaining the dental biofilms (plaques) or dental calculus samples from volunteers' is very simple, painless and noninvasive process. Each volunteer should agree in writing to take samples and provide the data in the anamnestic questionnaire. Volunteers can be sorted based on their life style e.g.: vegetarians, smokers, based on health problems etc. The selection of volunteers should be based on the targeted microbiota from the dental biofilm e.g. autochthonous or allochthonous or obtaining of pathogenic bacteria from target places of niches in the oral cavity, e.g. caries, periodontal pocket in periodontitis, ulcer in stomatitis aphthosa or other lesions. For obtaining the autochthonous microbiota, volunteers starve overnight after carefully brushing their teeth. The sample of dental biofilm has to be obtained early in the morning immediately after waking up. Volunteers cannot eat, drink or brush their teeth before sampling. The composition of mixture of autochtonous and allochtonous microbiota depends on sampling time: morning, midday or night, whereas food does not present a problem. It is also necessary to have volunteers with similar dental care and similar food consumption habits, and also to select the concrete localization on the teeth, e.g.: incisor, premolar, molar teeth. The lingual area of maxillary incisors or the buccal area of the first molar are predicted places for obtaining the calculus. Both areas are near to ductus salivarius from sublingual and parotid glands. Components of saliva have important effect on calcification of dental plaque. For sampling this plaque, the sterile syringe needle and gloves are used. It is important to take calculus samples without injuring of the gum.

Microbiological cultivation methods

Samples of dental plaques obtained from volunteers are used for cultivation of viable bacteria in dental biofilm. Pieces of dental plaque (2x3mm) samples obtained with help of sterile syringe needle are added to 2 ml Eppendorf tube containing 200 µl of filtrated PBS. Than samples are vortexed 5 min on maximal speed and divided to 2 Eppendorf tubes at volume 100 µl. First Eppendorf tube filed with 1.5 ml of Brain Heart Infusion (BHI) (Merck K GaA Darmstadt, Germany) broth. Second Eppendorf tube contains 1.5 ml deMan, Rogosa and Sharpe (MRS)(CONDA S.A, Madrid Spain) broth. After 5 hrs broth cultivation at 37 °C, 100 µl of samples from BHI broth are inoculated simultaneously on Blood agar (Tryptic soy agar (TSA) with 5 % ram's blood (BBL, Microbiology Systems, Cockeysville, USA), MacConkey agar (MCC)(CONDA S.A. Madrid, Spain), M-Enterococcus agar (ME)(Decton Dickinson S.A, Le point delClaix France). Collections of the samples are cultivated in two sets. One set is used for aerobic cultivation during 24 hrs at 37 °C and the next one for anaerobic

cultivation with BD GasPak™ systems Becton, Dickinson and Company. Samples from MRS broth are inoculated on MRS agar at volume 100 µl after 48 hrs of cultivation. Each different bacterial colony from agars is selected and inoculated separately for subsequent processing. Selected strains are used for preparing of glycerol conserve, analyzed for catalase activity and with help of Gram staining microscopically.

Bacterial strains storage

Each bacterial colony cultivated in BHI broth is stored in autoclaved glycerol and sterile adequately broth in Eppendorf tube. Similar conserve is prepared of MRS broth for lactic acid bacteria. During the preparation of the stock, it is necessary to use sterile scissors for cutting the end of 1000 µl tip, because glycerol is viscous, and it is easy and quick to fill the tip and Eppendorf tube with glycerol. For prevention of contamination from pipette, it is better to use tips with filters (Grainer bio-one RNase DNase free). The same volume of predicted broth is added to glycerol filled Ependorf tube. Selected colonies are transferred to prepare Eppendorf tubes with sterile one-time used inoculation loops. It is also possible to use 200 µl tips (Grainer bio-one RNase DNase free) for this purpose. The glycerol stock should be marked with the name of the strain and the paper label fixed with transparent adhesive tape because low temperature (-70 °C) and the high % of humidity after refreezing can change adhesive characters of paper and it can be lost. After adding the colonies into a glycerol broth conserve, it is necessary to vortex it or shake the tubes using an orbital shaker for several minutes. Glycerol stock prepared this way can be stored at -70 °C several months. It is better to prepare a collection of each of the selected colonies from the tested groups with different growth characters and store it in glycerol stock for storage of selected bacterial strains and prepare fresh at least 24 hrs cultivated colonies before DNA isolation or/and MALDI-TOF Biotyper processing. Other option for storage of isolated bacterial strains is Microbank (Pro Lab Diagnostic) used according to the manufacturer's instructions.

Bacterial strains identification

A clear colony is selected for isolation of DNA by DNAzol Direct (Molecular Research Center Inc., Cincinnati, USA) according to the manufacturer's instructions. Subsequently 1µl of DNA samples are added to One Taq 2X Master Mix (New England BioLabs) and amplified by PCR methods used for detection of 16S ribosomal RNA (rRNA) genes by using universal primers: 27F (5-AGAGTTTGATCMTGGCTCAG-3) and 1492R (5-CGGYTACCTTGTTACGACTT-3). Cycling conditions are: 5 min hot start at 94 °C, 31 cycles of 1 min at 94 °C, 1 min at 55 °C and 3 min at 72 °C and a final 10 min extension step at 72 °C (TProfesional Basic, Biometra GmbH, Göttingen, Germany). Aliquots of the PCR products are separated by horizontal 0.7 % agarose gel electrophoresis in TAE buffer (pH 7.8). The gel was stained with GelRed™ (Biotium Inc., Hayward, USA) and visualized under UV light. Products of amplification are purified by NucleoSpin® Gel and PCR Clean-Up Kit (Mancherey-Nagel GmbH & Co. KG, Düren, Germany) as per manufacturer's instructions. It is also possible to sending amplificates to sequence analysis, which obtain purification step before sanger sequencing. Clear sequencies from both directions are assembled and analyzed with Blast n analysis. Other quick identification possibilities are identification of selected clear colonies also by MALDI TOF Biotyper analysis (Bruker Daltonics, Bremen, Germany).

Identification of isolated strains by The Matrix-Assisted Laser Desorption Ionisation-Time of Flight (MALDI-TOF) Mass Spectrostrometry (MS) used for

One colony from the plate is added into the Eppendorf tube with 300 µl of distilled deionised H_2O and vortexed. Then, 900 µl of ethanol is added into the tube and vortexed again. The next step is centrifugation at 13000 RPM during 2 min and after that, the supernatant is discarded out. The pellet is dried in vacuum centrifuge air drier (Concentrator plus, Eppendorf). The dry pellet is mixed with 70 % formic acid (10 – 50 µl, depending on pellet's size) using pipette and vortex. After that, the same volume of acetonitrile is added into the mixture and vortex. The tube is centrifuged at 13000 RPM during 2 min. 1 µl of supernatant (from 0.5 to 2 µl) is added to MALDI-TOF target plate and dried. After drying, 1 µl of MALDI-TOF matrix solution is added. The bacterial strains are identified with MALDI-TOF MS and BioTyper 2.0 systems (Bruker Daltonics, Bremen, Germany).

Identification of isolated strains of streptococcus mutans, str. salivarius, str. oralis, lactobacillus spp., lb. reuteri, lb. paracasei and their glucosyl or fructosyl transferaze genes by PCR

Table 1: PCR methods useful for identification of oral bacterial Streptococcus or Lactobacillus strains

Species/ Target gen	Primers	PCR protocol	Product size	Literature
Str. mutans Glycosyltrans-ferases gene (gtf)	MKD-F 5´GGCACCACAACATTGGGAAGCTCAGTT3 MKD-R 5´GGAATGGCCGCTAAGTCAACAGGAT3´	95 ºC, 13 min 30x [95°C, 30 sec, 67 ºC, 1 min, 72 ºC, 1 min] 72 ºC, 5 min	433 bp	[60] [61]
Str. salivarius Glycosyltrans-ferases gene (gtf)	MKK-F 5´GTGTTGCCACATCTTCACTCGCTTCG 3´ MKK-R 5´CGTTGATGTGCTTGAAAGGGCACCATT3´	95 ºC, 13 min 30x [95°C, 30 sec, 66 ºC, 1 min, 72 ºC, 1 min] 72 ºC, 5 min	544 bp	[60]
Str. oralis Glycosyltrans-ferases gene (gtf)	gtfR MKR-F 5´TCCCGGTCAGCAAACTCCAGCC3´ gtfR MKR-R	95 ºC, 13 min 30x [95°C, 30 sec, 66 ºC, 1 min, 72 ºC, 1 min] 72 ºC, 5 min	374 bp	[60]

	5´ GCAACCTTTGGATTTGCAAC3´			
Lactobacillus spp. Glycosyltrans-ferases gene (gtf)	DexreuV 5´GTGAAGGTAACTATGTTG3´ DexreuR : 5´ATCCGCATTAAAGAATGG3´	94 ºC, 5 min 31x [94 ºC, 1 min, 47ºC, 1 min, 72 ºC, 1 min] 72 ºC, 10min	600 bp	[62]
Lactobacillus reuteri	L-reu-1 5′ CAGACAATCTTTGATTGTTTAG3′ L-reu-4 5′GCTTGTTGGTTTGGGCTCTTC3′	95°C, 10 min 35x [95°C, 30 sec, 60°C, 30 min, 72°C, 1 min] 72°C, 10 min	303 bp	[63]
Lactobacillus fermentum	PLFf 5′GTTGTTCGCATGAACAACGCTTAA3′ PLFr 5´CGACGACCATGAACCACCTGT3′	95°C, 10 min 35x [95°C, 30 sec, 65°C, 30 sec, 72°C, 1 min] 72°C, 10 min	889 bp	[64]
Lactobacillus plantarum	PLPf 5′ATGAGGTATTCAACTTATG3′ PLPfr 5′GCTGGATCACCTCCTTTC3′	95°C, 10 min 35x [95°C, 30 sec, 65°C, 30 sec, 72°C, 1 min] 72°C, 10 min	280 bp	[65]

Other PCR condition for detection of other group of lactobacilli is mentioned in publication [66].

Methods used for isolation of oral potential beneficial bacteria

Potential probiotic bacteria, like Streptococcus salivarius, can be successfully isolated by a simple method, using 24-hour pre-cultivation of dental biofilm samples in Brain hearth infusion medium (BHI) (Merck K GaA Darmstadt, Germany) at 37 °C, and subsequent 24-hour anaerobic cultivation (BBL GasPak Plus ™, BD, USA) on autoclaved M Enterococcus Agar (Decton Dickinson S.A, Le point delClaix France) at 37 ° C. Large transparent milky S-form colonies has grown on the agar. Using a microscope, one can observe bacteria of G+ cocci shapes, arranged into chains or tetra-cocci, which have considerably bigger diameter compared to the other streptococci. However, other bacteria can also grow on this medium but the genus Streptococcus salivarius is the most common strain (Figure 2).

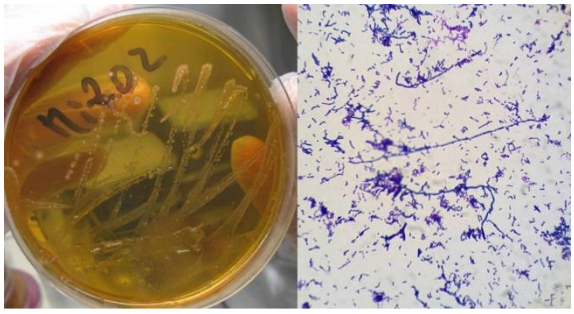

Figure 2: Streptococcus salivarius cultivated on M enterococcus agar plate

For isolation bacteria of the genus Lactobacillus, it appears that the most suitable way is to pre-incubate them in MRS liquid medium at 37 ° C for 24hr, and then cultivate them on deMan, Rogosa and Sharpe MRS agar (CONDA S.A, Madrid Spain) at 37 °C anaerobically for 48 hours (BBL GasPak Plus ™, BD, USA). The colonies are white, L-shaped, and under a microscope they appear as G+ long rods. However, the lactic acid bacteria may grow on this medium, such as some streptococci or yeasts, but the genus Lactobacillus is the most common (Fig. 4). For this reason, it is necessary to identify microscopically each different colony before its inoculation. A wide range of methods is available for identification, methods based on a biochemical profile, or molecular methods involving amplification of DNA from the obtained samples by universal primers in the PCR reaction and sequencing or using specific primers of each representative like in (Table 1). A rapid and sensitive MALDI-TOF Biotyper analysis can also be used for this purpose (Figure 3).

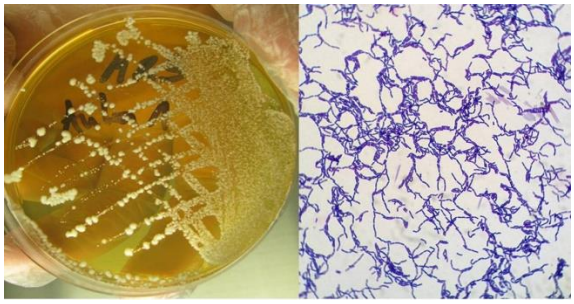

Figure 3: Lactobacillus spp. cultivated on MRS agar

The methods used for detection of spirochetal bacteria based on visualization of morpholocical characteristics of live or death bacteria in a fresh dental biofilm.

For visualization of uncultivable bacteria is used a principle of Viability fluorescent quick test on polycarbonate filters (VFQTOPF) method [67]. The samples isolated from the dental biofilm are kept in the sterile PBS at 37 °C. A 100 µl of the purified samples are incubated with 2.5 µl [1 mM] carboxyfluorescein diacetate (CFDA) during 20 min at 37 °C. 100 µl of the filtered samples are processed by vacuum filtration on surface of 25 mm Ø and 0.2 µm polycarbonate filters (Merck Millipore), where 50 µl of DAPI solution [1 mg/ml] is directly applied on the wet filter and incubated at room temperature during 10 min. The wet filters are moved on a glass slide, mounted with a drop of Vectashield Medium (Vector

Laboratories, Peterborough, UK) and a cover slide is fixed around edges with transparent nail polish. Then the slides are examined with epifluorescent microscopy in our case Carl Zeiss Axio Observer Z1 epifluorescence microscope with Filter Set 38H and 49 detecting the carboxyfluorescein CF and DAPI, respectively. Axio Vision Rel 4.8 software is used for the analysis of microphotography. This method VFQTOPF is used for visualization of no cultivable or hardly cultivable bacteria from samples of the dental biofilm. No exact identification of bacterial compounds presents limitation of this method. It is possible to check the dental plaque of hardly cultivated bacteria in samples, e.g. spirochetes (Figure 4). Detection of spirochetes namly Treponema denticola in tested samples is possible by PCR with help of primers: TAATACCGAATGTGCTCATTTACAT-3′ and 5′-TCAAAGAAGCATTCCCTCTTCTTC TTA-3 and with amplification conditions: 95 °C for 2 min, 36 cycles [at 95 °C for 30 s, a 60 °C for 1 min, at 72 °C for 1 min] and a final step of 72 °C for 2 min according [68].

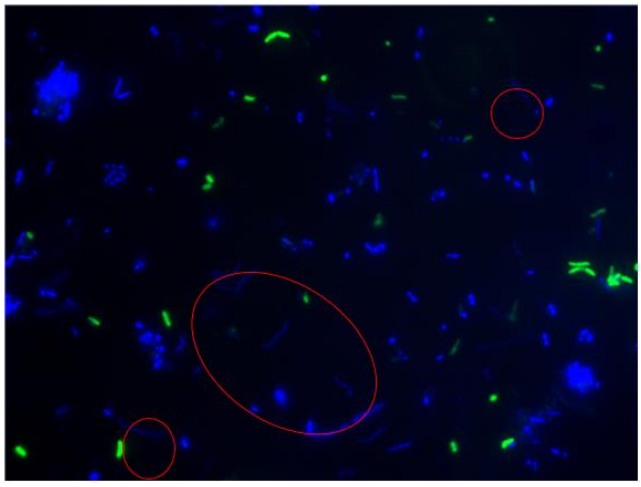

Figure 4: Different bacterial morphology detected in dental biofilm with VFQTOPF for example Spirochetes (red circle) detected by DAPI (blue middle left) or other Bacillus bacteria detected by CFDA which confirm their metabolic activity (green midle right).

The detection of production the bacteriocines salivaricin and reuterin by PCR methods

Many bacteria use bacteriocines, like strategies for surviving in biofilms. This substance is interesting also in search of probiotic potential, because their can kill or inhibit growth of pathogenic bacteria. Many of pathogenic bacteria have similar bacteriocin dependent strategies, like in case of Pseudomonas aeruginiosa or Escherichia coli [69, 70]. Potential benefitial oral microbiota can synthesize reuterin produced by Lactobacillus reuteri or salivaricine produced by Streptococcus salivarius [71, 72]. In case of salivaricine A it is easy to detect their presence and activity with help of growth inhibition of Micrococcus luteus like in case of see below concretely [73].

The PCR method for detection of salivaricin or reuterin genes

The isolation of DNA from lactobacilli strains is performed by the NucleoSpin® Tissue Macherey-Nagel kit using a lysis solution for heavier DNA isolates of bacteria during overnight incubation at 95 °C. The next steps are according to the manufacturer's procedure. DNA quality is better verified by Nanodrop spectrophotometric analysis. It is also possible to use one bacterial colony and 100 µl DNAzol direct and heat it of 95°C during 15min for isolation of DNA for this purpose, but storage of DNA samples for next analysis is time limited. The isolation steps are according to the manufacturer and specific sample. For robust PCR we can used Mastermix One taq2x MM (England Biolabs) and specific primers in concentration 33µMol at volume 0.6µl and 1µl of template DNA inadequately concentration or 1µl of DNA isolated with help of DNAzol direct. Conditions of PCR are described in (Table 2).

The disc diffusion method for Lactobacillus reuteri for testing of growth inhibition activity against pathogens

We recommended disc diffusion test for detection of the inhibitory properties of beneficial microorganisms. Selected lactobacilli strains are grown on MRS agar for 48 hr anaerobically at 37 °C (Gas Pak Plus, BBL, Microbiology Systems, Cockeysville, USA). Then prepare a standardized suspension with an optical density of 1 McFarland by dissolving several solitary colonies in 5 ml of physiological saline. Sterile clean discs (6 mm diameter, BBL, Cockeysville, USA) are placed on Petri dishes with 20 ml of PYG agar. The sterile discs are inoculated with 5 µl of each standardized suspension of lactobacilli. As a check, one Petri dish is served with a clean, uninoculated disc. The plates with discs are incubated for 48 hr anaerobically at 37 °C. The discs are removed with a sterile two siring needle or tweezer after incubation. Subsequently, 3 ml of 0.7% PYG agar is inoculated with 0.3 ml of the indicator pathogenic strain and put into lactobacilli plates. Pathogenic strains are firstly incubated for 18hr in PYG broth at 37 ° C. The plates with pathogen broth are incubated for 24hr aerobically at 37 °C. After incubation, we measure the diameter of the inhibition zones. The results are recorded in the table as the arithmetic mean of the three measurements ± standard deviation.

The first testing of Streptococcus salivarius inhibition is used the disc diffusion test with Micrococcus lutheus [73]. This test analyses the activity of the BLIS produced in agar and determines the activity spectrum of Sal9 producers. Briefly, the test strain is inoculated across the surface of the Blood agar medium in a glass Petri dish as a 1 cm-wide streak. After incubation, the strain growth is stopped by exposure of chloroform vapour for 30 min. The plate is then aired for 15 min before 24hr inoculating cultures as the indicator strains across the original tested strain. The plate is incubated for 24hr and examined for the zones of the indicator strain growth inhibition. The inhibition activity against the selected standard indicators was recorded in code form by considering the indicators as three triplets. The inhibition of the first member of a triplet is given a score of 4, the second a score of 2, and the third a score of 1. Absence of inhibitor action against an indicator is scored as 0. The code is recorded as a sequence of three numbers representing the sum of each triplet. All tests are performed in duplicate, and further testing is undertaken until consistency of the inhibition patterns is obtained [77].

Table 2: PCR conditions used for detection of genes coding production of bacteriocines: salivaricine and reuterin

Target gen	Primers	PCR protocol	Product size	Literature
salA (salivaricine)	SalAUS 5´GTAGAAAATATTTACTACATACT3` SalADS 5`GTTAAAGTATTCGTAAAACTGATG3`	95 ºC, 13 min 30x [95 ºC, 30 sec, 55 ºC, 1 min, 72 ºC, 1 min] 72 ºC, 5 min	338 bp	[73-75]
Lactobacillus reuteri glycerol dehydrogenase gldC (reuterin)	GD1f 5′GTTCAGTCCGCCGCATATC3′ GD1r 5′GCCGCTCTTCGTGGATTTC3′	94 °C, 5 min, 34x [94 °C, 1 min, 58 °C, 30 sec, 72 °C, 50 sec] 72 °C, 7 min	562 bp	[76]

Conclusion

The isolation of potencial beneficial microbes from oral cavity for next deep research is the first step of preparing functional beneficial microbes, which can be used as prevention or also used for treatment of oral diseases. The MALDI-TOF Biotyper identification is a perfect tool for quick identifications of isolated dental biofilm strains, pathogenic or non-pathogenic. For better declaration of your identification, you can use the PCR methods and next sequencing of products. Isolates bacteria can be also identified with biochemical or serological methods. The mechanisms of probiotic action are associated with resistance to colonisation and immunomodulation [27], adherence of bacteria and interspecies interactions [27, 78]. During recent years there has occurred a shift towards ecological and microbial community-based approach to the therapy of oral cavity diseases. With the increasing resistance to antibiotics, the use of probiotics appears as a prospective alternative treatment or preventative measure in the control of these diseases. From the clinical point of view, it is not yet possible to give direct recommendations for the use of probiotics. However, the available scientific evidence indicates that probiotic therapy is a promising approach also in the field of stomatology. The potential beneficial strains of Streptococcus salivarius or Lactobacillus reuteri and others bacterial strains isolated from many oral biofilms can be selected for next research based on their production of bacteriocines and on growth inhibition level against oral pathogenic bacteria not only in human but also in social animals like dogs and cats.

Acknowledgements

This study was carried out with financial support of the operation program Research and Development within the project, VEGA 1/0081/17, Study of the effect of beneficial microorganisms and their bioactive products on the inhibition of biofilm-forming pathogens and by the project Medical University Park in Košice (MediPark, Košice) ITMS:26220220185 supported by Operational Programme Research and Development (OP VaV-2012/2.2/08-RO) (Contract No. OPVaV/12/2013).

References

1. Sampaio-Maia B, F Monteiro-Silva (2014) Acquisition and maturation of oral microbiome throughout childhood: An update. *Dent Res J (Isfahan)* 11: 291-301. [Crossref]
2. Chandki R, Banthia P, Banthia R (2011) Biofilms: A microbial home. *J Indian Soc Periodontol* 15: 111-114. [Crossref]
3. Marsh PD (2005) Dental plaque: biological significance of a biofilm and community life-style. *J Clin Periodontol* 6: 7-15. [Crossref]
4. Jakubovics, NS (2015) Saliva as the Sole Nutritional Source in the Development of Multispecies Communities in Dental Plaque. *Microbiol Spectr*. [Crossref]
5. Huang R, Li M, Gregory RL (2011) Bacterial interactions in dental biofilm. *Virulence* 2: 435-444. [Crossref]
6. Jenkinson HF, RJ Lamont (2005) Oral microbial communities in sickness and in health. *Trends Microbiol* 13: 589-595. [Crossref]
7. Avila M, Ojcius DM, Yilmaz O (2009) The oral microbiota: living with a permanent guest. *DNA Cell Biol* 28: 405-411. [Crossref]
8. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE (2005) Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol* 43: 5721-5732. [Crossref]
9. Li J, Quinque D, Horz HP, Li M, Rzhetskaya M, et al. (2014) Comparative analysis of the human saliva microbiome from different climate zones: Alaska, Germany, and Africa. *BMC Microbiol* 14: 316. [Crossref]
10. Marsh PD, RS Percival (2006) The oral microflora--friend or foe? Can we decide? *Int Dent J* 56: 233-239. [Crossref]
11. Preza D, Olsen I, Willumsen T, Grinde B, Paster BJ (2009) Diversity and site-specificity of the oral microflora in the elderly. *Eur J Clin Microbiol Infect Dis* 28: 1033-1040. [Crossref]
12. Yoon Lee (2013) Diagnosis and Prevention Strategies for Dental Caries. *J Lifestyle Med* 3: 107-109. [Crossref]
13. Moynihan P (2016) Sugars and Dental Caries: Evidence for Setting a Recommended Threshold for Intake. *Adv Nutr* 7: 149-156. [Crossref]
14. Lemos JA, Quivey RG Jr, Koo H, Abranches J (2013) Streptococcus mutans: a new Gram-positive paradigm? *Microbiology* 159: 436-445. [Crossref]
15. Kianoush, N, Adler CJ, Nguyen KA, Browne GV, Simonian M, et al. (2014) Bacterial profile of dentine caries and the impact of pH on bacterial population diversity. *PLoS One* 9: e92940. [Crossref]
16. Silva N, Abusleme L, Bravo D, Dutzan N, Garcia-Sesnich J, et al. (2015) Host response mechanisms in periodontal diseases. *J Appl Oral Sci* 23: 329-355. [Crossref]
17. Zahradnik RT, Magnusson I, Walker C, McDonell E, Hillman CH, et al. (2009) Preliminary assessment of safety and effectiveness in humans of

ProBiora3, a probiotic mouthwash. *J Appl Microbiol* 107: 682-690. [Crossref]

18. Torrungruang K, Jitpakdeebordin S, Charatkulangkun O, Gleebbua Y (2015) Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Treponema denticola / Prevotella intermedia Co-Infection Are Associated with Severe Periodontitis in a Thai Population. *PLoS One* 10: e0136646. [Crossref]
19. Atanasova KR, Yilmaz O (2015) Prelude to oral microbes and chronic diseases: past, present and future. *Microbes Infect* 17: 473-483. [Crossref]
20. Li X, Kolltveit KM, Tronstad L, Olsen I (2000) Systemic diseases caused by oral infection. *Clin Microbiol Rev* 13: 547-558. [Crossref]
21. Grudianov AI, Dmitrieva NA, Fomenko EV (2002) [Use of probiotics Bifidumbacterin and Acilact in tablets in therapy of periodontal inflammations]. *Stomatologiia (Mosk)* 81: 39-43. [Crossref]
22. Reid G, Dols J, Miller W (2009) Targeting the vaginal microbiota with probiotics as a means to counteract infections. *Curr Opin Clin Nutr Metab Care* 12: 583-587. [Crossref]
23. Borchert D, Sheridan L, Papatsoris A, Faruquz Z, Barua JM, et al. (2008) Prevention and treatment of urinary tract infection with probiotics: Review and research perspective. *Indian J Urol* 24: 139-144. [Crossref]
24. Krutmann J (2009) Pre- and probiotics for human skin. *J Dermatol Sci* 54: 1-5. [Crossref]
25. Burton JP, Drummond BK, Chilcott CN, Tagg JR, Thomson WM, et al. (2013) Influence of the probiotic Streptococcus salivarius strain M18 on indices of dental health in children: a randomized double-blind, placebo-controlled trial. *J Med Microbiol* 62: 875-884. [Crossref]
26. Zambori C, Morvay AA, Sala C, Licker M, Gurban C, et al. (2016) Antimicrobial effect of probiotics on bacterial species from dental plaque. *J Infect Dev Ctries* 10: 214-221. [Crossref]
27. Meurman JH (2005) Probiotics: do they have a role in oral medicine and dentistry? *Eur J Oral Sci* 113: 188-196. [Crossref]
28. Anirban Chatterjee, Hirak Bhattacharya, Abhishek Kandwal (2011) Probiotics in periodontal health and disease *J Indian Soc Periodontol* 15: 23-28. [Crossref]
29. Bizzini B, Pizzo G, Scapagnini G, Nuzzo D, Vasto S (2012) Probiotics and oral health. *Curr Pharm Des* 18: 5522-5531. [Crossref]
30. Haukioja A (2012) Probiotics and oral health. *Eur J Dent* 4: 348-355. [Crossref]
31. Mahasneh SA, Mahasneh AM (2017) Probiotics: A Promising Role in Dental Health. *Dent J (Basel)*. [Crossref]
32. Haukioja A, Loimaranta V, Tenovuo J (2008) Probiotic bacteria affect the composition of salivary pellicle and streptococcal adhesion in vitro. *Oral Microbiol Immunol* 23: 336-343. [Crossref]
33. James SM, JR Tagg (1991) The prevention of dental caries by BLIS-mediated inhibition of mutans streptococci. *N Z Dent J* 87: 80-83. [Crossref]
34. Doyle H, Pierse N, Tiatia R, Williamson D, Baker M, et al. (2018) The Effect of the Oral Probiotic Streptococcus salivarius (K12) on Group A Streptococcus Pharyngitis: A Pragmatic Trial In Schools. *Pediatr Infect Dis J* 37: 619-623. [Crossref]
35. Allaker RP, Stephen AS (2017) Use of Probiotics and Oral Health. *Curr Oral Health Rep* 4: 309-318. [Crossref]
36. Oldak A, et al. (2017) Comparison of Antibacterial Activity of Lactobacillus plantarum Strains Isolated from Two Different Kinds of Regional Cheeses from Poland: Oscypek and Korycinski Cheese. *Biomed Res Int* 2017: 6820369.
37. Koll P, Mändar R, Marcotte H, Leibur E, Mikelsaar M, et al. (2008) Characterization of oral lactobacilli as potential probiotics for oral health. *Oral Microbiol Immunol* 23: 139-147. [Crossref]
38. Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, et al. (2004) Lactobacillus reuteri in bovine milk fermented decreases the oral carriage of mutans streptococci. *Int J Food Microbiol* 95: 219-223. [Crossref]
39. Mortazavi S, Akhlaghi N (2012) Salivary Streptococcus mutans and Lactobacilli levels following probiotic cheese consumption in adults: A double blind randomized clinical trial (*). *J Res Med Sci* 17: 57-66. [Crossref]
40. Mayanagi G, Kimura M, Nakaya S, Hirata H, Sakamoto M, 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. [Crossref]
41. Shimauchi H, Mayanagi G, Nakaya S, Minamibuchi M, Ito Y, 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. [Crossref]
42. Suzuki N, Yoneda M, Tanabe K, Fujimoto A, Iha 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. [Crossref]
43. Iwamoto T, Suzuki N, Tanabe K, Takeshita T, Hirofuji T (2010) Effects of probiotic Lactobacillus salivarius WB21 on halitosis and oral health: an open-label pilot trial. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 110: 201-208. [Crossref]
44. Strahinic I, Busarcevic M, Pavlica D, Milasin J, Golic N, et al. (2007) Molecular and biochemical characterizations of human oral lactobacilli as putative probiotic candidates. *Oral Microbiol Immunol* 22: 111-117. [Crossref]
45. Matsumoto M, Tsuji M, Sasaki H, Fujita K, Nomura R, et al. (2005) Cariogenicity of the probiotic bacterium Lactobacillus salivarius in rats. *Caries Res* 39: 479-483. [Crossref]
46. Almståhl A, Carlén A, Eliasson L, Lingström P (2010) Lactobacillus species in supragingival plaque in subjects with hyposalivation. *Arch Oral Biol* 55: 255-259. [Crossref]
47. Byun R, Nadkarni MA, Chhour KL, Martin FE, Jacques NA, et al. (2004) Quantitative analysis of diverse Lactobacillus species present in advanced dental caries. *J Clin Microbiol* 42: 3128-3136. [Crossref]
48. Colloca ME, Ahumada MC, López ME, Nader-Macías ME (2000) Surface properties of lactobacilli isolated from healthy subjects. *Oral Dis* 6: 227-233. [Crossref]
49. Kõll-Klais P, Mändar R, Leibur E, Marcotte H, Hammarström L, et al. (2005) Oral lactobacilli in chronic periodontitis and periodontal health: species composition and antimicrobial activity. *Oral Microbiol Immunol* 20: 354-361. [Crossref]
50. Barbour A, K Philip (2014) Variable characteristics of bacteriocin-producing Streptococcus salivarius strains isolated from Malaysian subjects. *PLoS One* 9: e100541. [Crossref]
51. Burton JP, Wescombe PA, Macklaim JM, Chai MH, Macdonald K, et al. (2013) Persistence of the oral probiotic Streptococcus salivarius M18 is dose dependent and megaplasmid transfer can augment their bacteriocin production and adhesion characteristics. *PLoS One* 8: e65991. [Crossref]
52. Tagg, JR (2009) Streptococcal Bacteriocin-Like Inhibitory Substances: Some Personal Insights into the Bacteriocin-Like Activities Produced by Streptococci Good and Bad. *Probiotics Antimicrob Proteins* 1: 60-66. [Crossref]
53. Santagati M, Scillato M, Patanè F, Aiello C, Stefani S (2012) Bacteriocin-producing oral streptococci and inhibition of respiratory pathogens. *FEMS Immunol Med Microbiol* 65: 23-31. [Crossref]
54. Di Pierro F, Colombo M, Giuliani MG, Danza ML, Basile I, et al. (2016) Effect of administration of Streptococcus salivarius K12 on the occurrence of streptococcal pharyngo-tonsillitis, scarlet fever and acute otitis media in 3 years old children. *Eur Rev Med Pharmacol Sci* 20: 4601-4606. [Crossref]
55. Guglielmetti S, Taverniti V, Minuzzo M, Arioli S, Stuknyte M, et al. (2010) Oral bacteria as potential probiotics for the pharyngeal mucosa. *Appl Environ Microbiol* 76: 3948-3958. [Crossref]
56. Cosseau, C, Devine DA, Dullaghan E, Gardy JL, Chikatamarla A, et al. (2008) The commensal Streptococcus salivarius K12 downregulates the innate immune responses of human epithelial cells and promotes host-microbe homeostasis. *Infect Immun* 76: 4163-4175. [Crossref]

57. Burton JP, Chilcott CN, Wescombe PA, Tagg JR, et al. (2010) Extended Safety Data for the Oral Cavity Probiotic Streptococcus salivarius K12. *Probiotics Antimicrob Proteins* 2: 135-144. [Crossref]
58. Burton JP, Chilcott CN, Moore CJ, Speiser G, Tagg JR (2006) A preliminary study of the effect of probiotic Streptococcus salivarius K12 on oral malodour parameters. *J Appl Microbiol* 100: 754-764. [Crossref]
59. Ishijima SA, Hayama K, Burton JP, Reid G, Okada M, et al. (2012) Effect of Streptococcus salivarius K12 on the in vitro growth of Candida albicans and its protective effect in an oral candidiasis model. *Appl Environ Microbiol* 78: 2190-2199. [Crossref]
60. Hoshino T, Kawaguchi M, Shimizu N, Hoshino N, Ooshima T, et al. (2004) PCR detection and identification of oral streptococci in saliva samples using gtf genes. *Diagn Microbiol Infect Dis* 48: 195-199. [Crossref]
61. Al-Ahmad A, Auschill TM, Braun G, Hellwig E, Arweiler NB (2006) Overestimation of Streptococcus mutans prevalence by nested PCR detection of the 16S rRNA gene. *J Med Microbiol* 55: 109-113. [Crossref]
62. Tieking M, Kaditzky S, Valcheva R, Korakli M, Vogel RF, et al. (2005) Extracellular homopolysaccharides and oligosaccharides from intestinal lactobacilli. *J Appl Microbiol* 99: 692-702. [Crossref]
63. Dommels YE, Kemperman RA, Zebregs YE, Draaisma RB, Jol A, et al. (2009) Survival of Lactobacillus reuteri DSM 17938 and Lactobacillus rhamnosus GG in the human gastrointestinal tract with daily consumption of a low-fat probiotic spread. *Appl Environ Microbiol* 75: 6198-6204. [Crossref]
64. Ruiz-Moyano S, Martín A, Benito MJ, Hernández A, Casquete R, et al. (2011) Application of Lactobacillus fermentum HL57 and Pediococcus acidilactici SP979 as potential probiotics in the manufacture of traditional Iberian dry-fermented sausages. *Food Microbiol* 28: 839-847. [Crossref]
65. Berthier F, Ehrlich SD (1998) Rapid species identification within two groups of closely related lactobacilli using PCR primers that target the 16S/23S rRNA spacer region. *FEMS Microbiol Lett* 161: 97-106. [Crossref]
66. Walter J, Tannock GW, Tilsala-Timisjarvi A, Rodtong S, Loach DM, et al. (2000) Detection and identification of gastrointestinal Lactobacillus species by using denaturing gradient gel electrophoresis and species-specific PCR primers. *Appl Environ Microbiol* 66: 297-303. [Crossref]
67. Popper M, Gancarčíková S, Maďar M, Mudroňová D, Hrčková G, et al. (2016) Amoxicillin-clavulanic acid and ciprofloxacin-treated SPF mice as gnotobiotic model. *Appl Microbiol Biotechnol* 100: 9671-9682. [Crossref]
68. Siqueira JF Jr, Rôças IN, Favieri A, Oliveira JC, Santos KR (2001) Polymerase chain reaction detection of Treponema denticola in endodontic infections within root canals. *Int Endod J* 34: 280-284. [Crossref]
69. Michel-Briand Y, Baysse C (2002) The pyocins of Pseudomonas aeruginosa. *Biochimie* 84: 499-510. [Crossref]
70. Kleanthous C (2010) Swimming against the tide: progress and challenges in our understanding of colicin translocation. *Nat Rev Microbiol* 8: 843-848.
71. Barbour A, Tagg J, Abou-Zied OK, Philip K (2016) New insights into the mode of action of the lantibiotic salivaricin B. *Sci Rep* 6: 31749. [Crossref]
72. Fujiwara N, Murakami K, Nakao M, Toguchi M, Yumoto H, et al. (2017) Novel reuterin-related compounds suppress odour by periodontopathic bacteria. *Oral Dis* 23: 492-497. [Crossref]
73. Barbour A, Philip K, Muniandy S (2013) Enhanced production, purification, characterization and mechanism of action of salivaricin 9 lantibiotic produced by Streptococcus salivarius NU10. *PLoS One* 8: e77751. [Crossref]
74. Wescombe PA, Upton M, Dierksen KP, Ragland NL, Sivabalan S, et al. (2006) Production of the lantibiotic salivaricin A and its variants by oral streptococci and use of a specific induction assay to detect their presence in human saliva. *Appl Environ Microbiol* 72: 1459-1466. [Crossref]
75. O'Shea EF, Gardiner GE, O'Connor PM, Mills S, Ross RP, et al. (2009) Characterization of enterocin- and salivaricin-producing lactic acid bacteria from the mammalian gastrointestinal tract. *FEMS Microbiol Lett* 291: 24-34. [Crossref]
76. Kiňová Sepová H, Bilková A (2013) Isolation and identification of new lactobacilli from goatling stomach and investigation of reuterin production in Lactobacillus reuteri strains. *Folia Microbiol (Praha)* 58: 33-38. [Crossref]
77. Wescombe PA, Upton M, Renault P, Wirawan RE, Power D, et al. (2011) Salivaricin 9, a new lantibiotic produced by Streptococcus salivarius. *Microbiology* 157: 1290-1299. [Crossref]
78. Stamatova I, Meurman JH (2009) Probiotics: health benefits in the mouth. *Am J Dent* 22: 329-338. [Crossref]