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Antimicrobial properties of new Curasept pastes and gels against pathogenic strains from red and yellow complexes present in the oral cavity

Właściwości przeciwdrobnoustrojowe nowych past i żeli Curasept na patogenne z kompleksów czerwonego i żółtego obecnych w jamie ustnej

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Keywords

MIC, MBC, oxidative stress, peridontitis, yellow and red complex

Summary

Introduction. The bacterial flora of the oral cavity is a very specific and diverse environment. There are 10^8 to 10^9 bacterial cells in 1 milliliter of saliva. The qualitative and quantitative composition of the salivary microflora is determined by the presence of microorganisms of individual ecological niches and is at the same time an indicator of oral health. The oral cavity contains saprophytic, potentially pathogenic, and sometimes pathogenic microorganisms. They live there permanently and temporarily, and can live with each other in symbiosis or antibiosis. The variables that affect the change in the oral microflora are saliva, which contains substances that inhibit the development of some microorganisms and the availability of food. The microorganisms living in the oral cavity need amino acids, proteins, carbohydrates, and glycoproteins in saliva to live and develop.

Aim. The aim of the study was to check the antibacterial properties of five different compunds recommended by Indent – a leader in dental prophylaxis and distributor of companies such as Curasept, Tello, Frezyderm which help accelerate healing and prevent persistent infections or recurrent canker sores.

Material and methods. The reference bacterial strains of probiotics strains as control *Bifidobacterium lactis* HNO 19, *Kluyveromyces marxianus fragilis* B0399, *Lactobactilius reuterii Prodentis*, *Lactobacillus salivarius* SGL03 and pathogenic strains of red and yellow complexes: *Porphyromonas gingivalis* (ATCC 33277), *Treponema denticola* (ATCC 35405), *Tanarella forsythia* (ATCC 43037), *Streptococcus sanguis* (ATCC 10556), *Streptococcus mutans* (ATCC 25175) were provided from LGC Standards U.K. and were used according to the recommendation of ISO 11133:2014. These strains were used to test antibacterial activity with analyzed compounds by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Results. The tested five compounds, showed super-selectivity an antimicrobial activity all analyzed bacterial strains of red and yellow complexes profile similar to that obtained with currently used antibiotics such as ciprofloxacin, bleomycin, and cloxacillin observed in MIC and MBC tests.
Conclusions. It should also be noted that the cost of the compounds obtained is low, what may be an attractive alternative to the currently used antimicrobial agents.

Słowa kluczowe

MIC, MBC, stres oksydacyjny, zapalenie przyzębia, kompleks żółty i czerwony

Streszczenie

Wstęp. Flora bakteryjna jamy ustnej jest bardzo specyficznym i zróżnicowanym środowiskiem. W 1 mililitrze śliny znajduje się od 10^8 do 10^9 komórek bakteryjnych. Skład jakościowy i ilościowy mikroflory śliny jest determinowany przez obecność mikroorganizmów poszczególnych nisz ekologicznych i jest jednocześnie wskaźnikiem zdrowia jamy ustnej. Jama ustna zawiera mikroorganizmy saprofityczne, potencjalnie patogenne, a czasami patogenne. Żyją tam na stałe i czasowo, mogą żyć ze sobą w symbiozie lub antybiozie. Zmiennymi, które wpływają na zmianę mikroflory jamy ustnej, są ślina, która zawiera substancje hamujące rozwój niektórych mikroorganizmów i dostępność pożywienia. Mikroorganizmy żyjące w jamie ustnej potrzebują aminokwasów, białek, węglowodanów i glikoprotein w ślinie, aby żyć i się rozwijać.
Cel pracy. Celem badania było sprawdzenie właściwości antybakteryjnych pięciu różnych związków rekomendowanych przez Indent – lidera w profilaktyce stomatologicznej i dystrybutora takich firm, jak: Curasept, Tello, Frezyderm, które pomagają przyspieszyć gojenie i zapobiegają przewlekłym infekcjom lub nawracającym aftom.
Materiał i metody. Referencyjne szczepy bakterii probiotycznych jako kontrola *Bifidobacterium lactis* HNO 19, *Kluyveromyces marxianus fragilis* B0399, *Lactobactilius reuterii Prodentis*, *Lactobacillus salivarius* SGL03 oraz patogenne szczepy kompleksów czerwonego i żółtego: *Porphyromonas gingivalis* (ATCC 33277), *Treponema denticola* (ATCC 35405), *Tanarella forsythia* (ATCC 43037), *Streptococcus sanguis* (ATCC 10556), *Streptococcus mutans* (ATCC 25175) zostały dostarczone przez LGC Standards U.K. i były używane zgodnie z zaleceniami normy ISO 11133:2014. Szczepy te zostały użyte do przetestowania aktywności przeciwbakteryjnej z analizowanymi związkami przy użyciu minimalnego stężenia hamującego (MIC) i minimalnego stężenia bakteriobójczego (MBC).
Wyniki. Przebadane pięć związków wykazało superselektywność i aktywność przeciwdrobnoustrojową, wszystkie analizowane szczepy bakteryjne kompleksów czerwonych i żółtych miały profil podobny do uzyskanego przy użyciu obecnie stosowanych antybiotyków, takich jak: cyprofloksacyna, bleomycyna i kloksacylina, obserwowanych w testach MIC i MBC.
Wnioski. Należy również zauważyć, że koszt uzyskanych związków jest niski, co może stanowić atrakcyjną alternatywę dla obecnie stosowanych środków przeciwdrobnoustrojowych.

Introduction

The microflora in a healthy oral cavity is in constant biological balance – homeostasis, it is called sessile or endogenous microflora. This situation changes when diseases occur in the body or when so-called exogenous or transient flora (acquired microflora) enters the oral cavity. This happens, for example, with weakened immunity (AIDS, chemotherapy), dehydration, systemic diseases, cancers, nutritional deficiencies (lack of iron, proteins, vitamins), hormonal disorders (during puberty, pregnancy, diabetes), antibiotic therapy, in childhood and old age. The oral environment with sessile microflora constitutes a so-called closed ecosystem, which includes microenvironments called ecological niches, such as: mouth and lips, buccal epithelium, tongue surface, supragingival surfaces of teeth, subgingival surfaces of teeth, epithelium of crevices and pockets, and saliva (1-6). Balance disorders may indicate disease processes occurring in the oral cavity (1-10).

The fluids flowing from the gingival crevices are an abundant source of nutrients for bacteria. Another endogenous generator of nutrients are substances released by the decaying periodontal tissues. This decomposition is enabled by hydrolytic enzymes, i.e. protease, collagenase, and hyaluronidase produced by the bacteria of the oral microflora.

Carbohydrates are an important source of energy for the bacteria living in the oral cavity. They are converted into glucans and fructans (1-12). Changing the environment in which bacteria live in the oral cavity can cause saprophytic bacteria to become pathogenic. The composition of the oral bacterial flora can be changed by drugs: cytostatics, steroids, bactericidal and bacteriostatic agents. The latter two groups of drugs inhibit the growth of bacteria, thus facilitating the growth of fungi and bacteria resistant to their action (2-5, 11, 13).

Physiologically, the bacterial flora of the oral cavity is diverse and consists of bacteria, fungi, mycoplasmas, protozoa and viruses (the most common is the herpes simplex virus, HSV). About 350 species of microorganisms have been cultured from the oral cavity, but many species have not yet been identified. Bacteria constitute the most numerous group. A significant proportion of bacteria living in the oral cavity do not exist in ecosystems other than the human body (4-6, 14-17). The processes of gradual colonization of ecological niches by bacteria in the oral cavity are called ecological succession. Over time, with the creation of new niches, the influx of new microorganisms, and the emergence of new environmental conditions, the ecosystem achieves stabilization - homeostasis. This state is called the finale of development, or the culmination point of the ecosystem (2-5, 11, 13).

The order of microbial colonization depends on the availability of nutrients and the breaking of the natural limit of non-specific immunity. The first to inhabit the oral cavity are the so-called pioneer species transmitted by the macroorganism and obtained from the environment. They colonize specific ecological niches and, over time, multiply, creating ecological communities. The environment in which they live changes as a result of their metabolic activity, which facilitates the penetration of other bacterial species into them, and thus begins ecological succession, which leads to a large and significantly diversified environment of sessile microflora, or to pathological changes (2-13). Each introduction of a new non-bacterial element into the oral cavity environment, from the eruption of the first tooth to complete dentures, contributes to the creation of new environmental conditions. In the new conditions, microorganisms develop rapidly, creating new ecological communities. The first to develop is the aerobic bacterial flora, which causes a decrease in the oxidation-reduction potential of a given niche, which in turn creates favorable conditions for the development of anaerobic bacteria (14-17).

The colonization of the tooth surface by microorganisms is possible due to adhesion or adherence processes. Adhesins enable the connection of the lipophilic surface of bacterial cells with the hydrophobic surface of epithelial cells. Adhesins are proteins that bind to carbohydrates using structures called fimbriae (fibrils of various lengths found on the cell surface). Fimbriae have been detected on streptococci – *S. anginosus*, *S. salivarius*, *S. oralis*, *S. mutans*, on *Actinomycetes* – *A. viscosus*, *A. naeslundi* and in Gram-negative rods – *Prevotella*, *P. intermedia*, *P. oralis*, *P. bucceae*, *P. melaniogenica* and the species *Porphyromonas gingivalis* (18-20).

Other adhesive substances are substances that stick together or transport substrates, e.g. polypeptides that bind salivary glycoproteins to bacterial cells or calcium ions. Extracellular polypeptides that react with streptococcal proteins behave similarly. The cell walls of streptococci contain lipoteichoic acids that can bind to the acquired salivary sheath (a thin layer that forms from salivary glycoproteins on teeth just a few minutes after brushing and adheres to them). An important role in colonization processes is played by cytoplasmic membrane polyproteins that act as a "transporter" of components, e.g. sugar or peptides associated with the acquired salivary sheath or the surfaces of other bacterial cells. So-called ligands take part in the adhesion processes – negatively charged salivary glycoproteins that can occur on epithelial cells of the mucous membrane, hard tissues of the tooth above and below the gums and on prosthetic restorations. These compounds are positively charged (Ca+2), so they bind to negatively charged bacterial adhesins of streptococci – *S. mutans*, *S. oralis*, *Actinomyces* rods, Gram-negative bacteria, i.e. *P. intermedia*, *F. nucelatum*, *P. gingivitis*, *E. corrdens* (2-13, 18-20).

Another important adhesion mechanism is bacterial aggregation, which involves the attachment of microorganisms to each other and their adherence to various surfaces. The main substrates in the oral cavity are: the acquired membrane on the hard surfaces of enamel and root cement (bones) and on the oral mucosa. The membrane covering the surfaces is about 1 micron thick and is characteristic of each surface. The membranes on the surfaces of hard tissues are not identical; on the epithelium they are referred to as the mucosal membrane (5, 13, 20-33).

Another important phenomenon in the colonization process is the extracellular polymers produced by streptococci from the S group, mutans. They are produced only in the presence of sucrose and aggregate very quickly on smooth enamel surfaces. A similar situation occurs among bacteria from the Actinomyces genus, but they colonize on the subgingival surfaces of the tooth. These processes initiate the formation of dental plaque, which results in carious changes (2-5, 13, 22-24). In the oral cavity, bacteria group into specific groups, also known as complexes, thanks to which they use nutrients better and defend themselves more effectively against the defense mechanisms of the macroorganism (32, 33). The concept of "bacterial complex" was introduced by Socransky (34). The division criterion is the relationship between bacteria. Socransky divided pathogens in the biofilm into 6 groups, to which he assigned an appropriate color – blue, yellow, green, purple, orange and red. The last three complexes (purple, orange and red) dominate in the subgingival space (34-40). In our studies, we focused on bacteria of the red and yellow complexes (fig. 1).

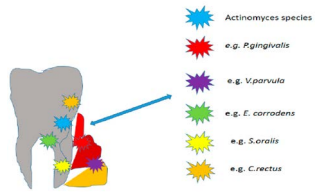

Fig. 1. Bacterial complexes in the oral cavity according to Socransky (34)

The yellow group consists of gram-positive bacteria, mainly streptococci (*Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus sanguis*). Purulent streptococci are characteristic of primary infections of the dental pulp and dentin (endodontium), where they penetrate individually or in the form of coaggregates into the dentinal tubules and are a common cause of caries. Streptococci are also responsible for causing angina and wound infections in the oral cavity. They belong to the permanent physiological flora of the oral cavity, also in healthy people (up to 20% of people in society are their carriers) (fig. 2). In the case of gingivitis, tooth extraction or endodontic procedures, they can penetrate through damage to the vessel walls into the bloodstream and cause transient bacteremia and even endocarditis. In the case of reinfection, they constitute about 20% of all pathogenic bacteria of the oral cavity (34-46). The yellow group also includes organisms from the red complex groups, for example *Porphyromonas gingivalis* and strains of *Fusobacterium nucleatum*, *Prevotella intermedia*, as well as *Streptococci* such as *S. anginosus* and *Streptococcus mitis*, although the most common are *S. gordonii*, *S. anginosus* and *S. oralis*. The *Streptococcus anginosus* group includes the species *S. intermedius* and *S. constellatus*, which have the greatest ability to penetrate dentinal tubules. Less frequently encountered are *S. salivarius*, *S. sanguis* and *S. mutans*, which are responsible for the production of polysaccharides that can enter the dental canals during the treatment of endodontic infections (fig. 2).

The red complex is the most pathogenic for periodontium. It also contains bacteria that occur most frequently in infected root canals and in abscesses of dental origin. It includes the following bacteria: *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* (34). The red complex also includes bacteria that produce black pigments, spirochetes, enterococci, streptococci, bacteria from the Actinomyces and Lactobacillus families and fungi from the Candida family. Bacteria that produce black pigments are Gram-negative anaerobes from the *Porphyromonas* and Prevotella families. They are responsible for the occurrence of inflammation and symptoms of pain and swelling. *Porphyromonas gingivalis* are Gram-negative, facultatively anaerobic bacteria that have fimbriae with numerous adhesins, which allow them to attach to periodontal tissues and enable coaggregation with other bacterial species. Additionally, they induce a proinflammatory cytokine response. These strains produce exoenzymes – cysteine proteases with trypsin-like properties, which cause tissue destruction. The end products of *P. gingivalis* fermentation are acetic, propionic and butyric acid, as well as volatile sulfur compounds, which have a cytotoxic effect on host cells. In this way, they destroy tissue integrity and lead to local changes in the microenvironment of the periodontal pocket (fig. 2).

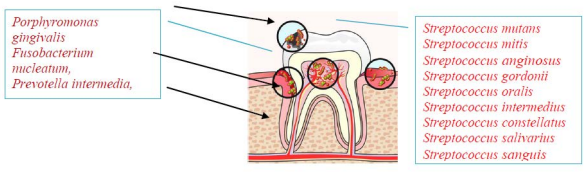

Fig. 2. Commensal bacteria with pathogenic activity of the yellow and red Sokranski syndrome

Aim

The aim of the study was to check the antibacterial properties of mouthwashes recommended by Indent – a leader in dental prophylaxis and distributor of companies such as Curasept, Tello, Frezyderm, which help accelerate healing and prevent persistent infections or recurrent canker sores.

Material and methods

Microorganisms and media

The reference bacterial strains of red and yellow complexes: *Porphyromonas gingivalis* (ATCC 33277), *Treponema denticola* (ATCC 35405), *Tanarella forsythia* (ATCC 43037), *Streptococcus sanguis* (ATCC 10556), *Streptococcus mutans* (ATCC 25175) and as a probiotic strains: *Bifidobacterium lactis* HNO 19, *Kluyveromyces marxianus fragilis* B0399, *Lactobactilius reuterii Prodentis*, *Lactobacillus salivarius* SGL03 were provided from LGC Standards (Manchester, U.K.) and growth media were used as described in Kucia (21) according to the recommendation of ISO 11133: 2014. These strains were used to test antibacterial activity test with analyzed compounds by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as described in (21, 47) and are show in supplementary materials in figure S1.

MIC and MBC tests

The MIC was estimated by a microtiter plate method using sterile 48 or 96-well plates (47). First, precursor and TIL solutions were prepared sterile deionized water at 20 mg mL-1. Fifty microliters of the solutions was placed in the first row of the plate. Next, 25 µL of sterile TSB medium was added to the other wells, and serial dilutions were performed. Then, 200 µL of inoculated TSB medium containing resazurin (0.02 mg mL^{-1}) as an indicator was added to all the wells. TSB medium was inoculated with 10(6) colony-forming units (CFU) mL^{-1} (approximately 0.5 McFarland units) of the bacterial strains. The plates were incubated at 30°C for 24 h. Color changes from blue to pink

or yellowish with turbidity were taken as positive, and the lowest concentration at which there was no visible color change was the MIC. The MBC was estimated based on the measurement of the dehydrogenases activity in the cultures after a 24-h incubation without the ILs. Four millilitres of a dense culture (approximately 10^9 CFU mL^{-1}) that was incubated for 24 h in TSB medium at 25°C was added to identical test tubes. Next, the tested compounds were added to the test tubes until the mixture reached final concentrations of 10-250 mg mL^{-1}. Then, the cultures containing the TILs were incubated for 1 h at 30°C. Next, 0.1 g of CaCO3 and 0.1 mL of a 3% triphenyltetrazolium chloride (TTC) solution were added to the test tubes. Then, the test tubes were sealed with parafilm and incubated for 1 h 30°C in darkness.

Statistical analysis

All experimental data from at least three different trials (n = 3) are given as means ± standard error of the mean (SEM, manufacturer, Saint Louis, MO, USA). To compare pairs of means, the Tukey post hoc test was used, indicating statistical significance with * $p < 0.05$,** $p < 0.1$, and *** $p < 0.01$ (10).

Analysed compounds – characteristics

All counds were used with manufacturer recommendation.

1. CURASEPT DAYCARE HERBAL INVASION − protective toothpaste with CPC-HAP 75 ml

Curasept Daycare Protection Booster toothpaste contains essential oils with innovative cetylpyridinium chloride and hydroxyapatite complex (CPC-HAP), whose synergistic action prevents the multiplication of microorganisms in the oral cavity for up to 4 hours after use. Contains a herbal flavor. It is SLS-free. Contains 900 ppm of fluoride. Fights caries and gum disease, halitosis, disinfects and remineralizes:

Active ingredients of Curasept Daycare Herbal Invasion toothpaste: 1) Cetylpyridinium chloride − has an antiseptic effect; 2) CPC-HA Complex (Cetylpyridinium Chloride + Hydroxyapatite) − hydroxyapatite creates a protective layer that remineralizes while gradually releasing cetylpyridine, providing extended protection for up to 4 hours; 3) Essential oils (menthol, eucalyptol, thymol, methyl salicylate) − prevent bacterial aggregation and slow down the growth of biofilm in the oral cavity. Penetrate the dental plaque and have a long-lasting effect at the supragingival and subgingival levels. They also help prevent gingivitis, help with daily mechanical plaque control and fight bad breath. Basic composition: Aqua, Sorbitol, Hydrated Silica, Xylitol, Propylene Glycol, Peg-32, Cellulose Gum, Cocamidopropyl Betaine, Hydroxyapatite, Calcium Glycerophosphate, Sodium Fluoride, Cetylopiyridinium Chloride, Calcium Phantothenate, 1,2-hexanediol, Potassium Nitrate, Cetraria Islandica Extract, Thymol, Elettaria Card amomum Seed Oil, Eucalyptol, Menthol, Methyl Salicilate, Stevia Rebaudiana Leaf/stem Extract, Sodium Saccharin, Aroma, Propanediol, Xanthan Gum, Phenoxyethanol, Ethylhexylglycerin, P-anisic Acid, Sodium Benzoate, Chlorphenesin, Tetrasodium Glutamate Diacetate, C.I. 19140, C.I. 42090.

2. CURASEPT ADS 100 − 1% chlorhexidine gum gel − 30 ml

The gum gel contains 1% chlorhexidine digluconate, which is one of the best ingredients on the market in effectively combating daily plaque. It also contains Purified water, Propylene Glycol, Hydroxy Ethyl Cellulose, PVP/VA copolymer, PEG-40 hydrogenated castor oil, Chlorhexidine digluconate, Sodium acetate, Aroma, Acetic acid, Sodium metabisulfite, Ascorbic acid.

Using the gel protects the gums and interdental spaces, and also prevents infection of the oral cavity by pathogens, which may be bacteria, fungi or viruses. In addition, the gel has a healing effect on ulcers in the oral cavity, treats periodontal disease, and also combats inflammation caused by irritation of the dental prosthesis. The ADS 100 gel in a 30 ml package is distinguished by its highly concentrated 1% chlorhexidine which acts radically against bacteria and dental plaque. It acts locally in the dental pocket for up to 12 hours after application. And also in the local treatment of gums during dental treatment, for owners of dentures, orthodontic appliances. It is also intended for patients with periodontal disease, having dental implants and also having a tendency to excessive accumulation of dental plaque. It does not change the taste of consumed food and drinks. The CURASEPT A.D.S. function slows down and inhibits the formation of bacterial plaque and prevents irritation of the gums and oral cavity.

3. CURASEPT PREVENT – toothpaste, 75 ml

Curasept Prevent is a toothpaste designed for prophylaxis and maintaining the health of gums and peri-implant tissues. Moreover, it helps maintain the effect of dental therapy in patients with chronic problems or during dental procedures. The synergistic selection of ingredients means that the paste helps maintain the ideal oral microflora. Additionally, Curasept Prevent paste hinders the multiplication of the most aggressive microorganisms, restores the balance of bacterial flora and improves the health of gum and periodontal tissues, while counteracting halitosis. CURASEPT PREVENT toothpaste is intended especially for: Diabetics, smokers, people suffering from metabolic diseases, with dry mouth, with weakened immunity, undergoing chemotherapy and radiotherapy, during orthodontic and implant treatment, patients (with complex prosthetic rehabilitation) treated for periodontal problems in the maintenance phase. Contains Sorbitol, Aqua, Hydrated Silica, Xylitol, PEG-32, Sodium Lauroyl Sarcosinate, Sodium Cocoyl Glutamate, VP-VA Copolymer, Cellulose Gum, Phenoxyethanol, Sodium Saccharin, Sodium Citrate, Sodium Benzoate, Hexetidine, Ozonized Olive Oil, Melaleuca Alternifolia Leaf Oil, Stevia Rebaudiana Leaf/Steam Extract, Colostrum, Aroma, C.I. 19140, C.i. 42090.

a) Ozonated Oil – Gradually releases ozone and creates a favorable micro-environment for maintaining the balance of oral microflora, as well as improving the health of tissues and mucous membrane.
b) Colostrum – Supports regeneration and strengthens gums and oral mucosa, thanks to the content of enzymes and proteins with a defensive effect that interact with lactoferrin and lysozyme.
c) PVP/VA – Thanks to its ability to adhere to teeth and mucous membrane, it creates a protective layer, hindering the adhesion and proliferation of bacterial plaque and delays the formation of biofilm.
d) Tea Tree Oil – Contains a natural set of substances with antioxidant action. Effectively prevents the formation of biofilm in the oral cavity.
e) Stevia – Natural sweetener with anti-caries effect. Widely used to inhibit the formation of biofilm in the oral cavity.

4. CURASEPT PREVENT – oral gel, 30 ml

Curasep Prevent is an oral gel indicated for the protection, prevention and maintenance of implants and control of risky situations related to periodontal disease. Thanks to its ability to adhere to teeth and mucous membrane, it creates a protective coating that inhibits the adhesion and proliferation of bacterial plaque and the formation of biofilm. Additionally, it improves the health of gum tissue, even in the case of comorbidities and balances the oral microflora.

Active ingredients of the gel:

a) Ozonated Oil – Gradually releases ozone and creates a favorable micro-environment for maintaining the balance of oral microflora, as well as improves the health of tissues and mucous membrane.
b) Colostrum – Supports regeneration and strengthens gums and oral mucosa, thanks to the content of enzymes and proteins with a defensive effect that interact with lactoferrin and lysozyme.
c) PVP/VA – Thanks to its ability to adhere to teeth and mucous membrane, it creates a protective layer, hindering the adhesion and proliferation of bacterial plaque and delays the formation of biofilm.
d) Tea tree oil – contains a natural set of substances with antioxidant action. Effectively prevents the formation of biofilm in the oral cavity.
e) Stevia – a natural sweetener with anti-caries action. Widely used to inhibit the formation of biofilm in the oral cavity.

Restores the balance of bacterial flora and improves the health of gum tissues, also in the case of comorbidities. Ideal for patients with implants, smokers, diabetics, with dry mouth and patients with weakened immune resistance. Prevents halitosis.

5. CURASEPT ADS 350 – office set of gels for gums with chlorhexidine 0.5%, 5 ml

The gel for gums contains chlorhexidine digluconate in a concentration of 0.5%, which is one of the best ingredients on the market in the effective fight against daily plaque. Using the gel protects the gums and interdental spaces, and also prevents infection of the oral cavity by pathogens, which may be bacteria, fungi or viruses. Additionally, the gel has a healing effect on ulcers in the oral cavity, treats gingivitis, and also combats inflammation caused by irritation of the dental prosthesis. Curasept ADS 350 gel is intended for patients after complicated extraction, after maxillofacial surgery, with post-bite trauma and mucosal damage with ulcers and aphthous stomatitis, with mucosal infections.

Contains A.D.S.® System – ANTI DISCOLORATION SYSTEM

Composition of the gel: Aqua, Propylene Glycol, Xylitol, Hydroxyethyl Cellulose, Chlorhexidine Digluconate, Ascorbic Amide. Peg-40 Hydrogenated Castor Oil, Sodium Metabisulphite, Aroma, Methylparaben.

Results

The effects of the 5 compounds investigated (illustrated in fig. 3-5) were evaluated within bacterial cells using a previously established methodology (21, 47). Following the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), the MIC values ranged between 0.25-2.5 µM, while the MBC values fell within the range of 1.5-2.5 (+/- 0.5) µM for analysed for four bacterials strains used as control and, Gram-negative strains and Staphylococcus, as depicted in figure 4. MIC serves as a reference for assessing the susceptibility or resistance of bacterial strains to the antibiotic applied in vitro. On the contrary, MBC represents the minimum concentration of an antibacterial agent required to eradicate bacteria, distinguishing it as bactericidal rather than bacteriostatic (34, 47). Basically, all compounds tested against Gram-positive and Gram-negative bacterial strains show good activity against them. And all MIC values are below 1.5 $\mu g/mL^{-2}$ (fig. 3). Interestingly, the change in the different compounds had a vital impact on activity. However, the fluorine compounds and other active ingredients was playing a role in the activity against some bacteria, which we confirmed with the activity of compound no. 1 and 5. This means that the compounds had a good potency compared to the analysed antibiotics (fig. 6). Interestingly, all five compounds showing broad spectrum activity against both Gram negative as well as gram positive bacteria. The steric hindrance created by the bulky fluorine atom and other active ingredients might prevent proper binding and interaction with the active site residues, thereby reducing the compound's overall efficacy. The analyzed probiotic strains were resistant to the action of pastes and gels, while the pathogenic strains were destroyed by strong cytotoxic properties damaging both gram-negative and gram-positive pathogens. This indicates their selective action on red and yellow complex bacteria.

Antimicrobial agents are often classified as either bacteriostatic or bactericidal. A medicine is deemed bactericidal if the MBC-MIC ratio is low (less than 1.5), and it is feasible

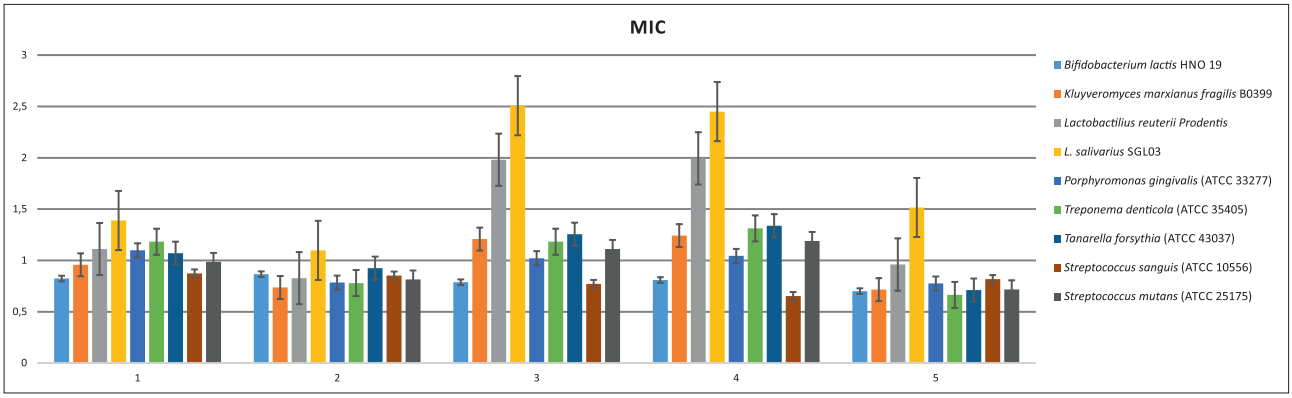

Fig. 3. Minimum inhibitory concentration (MIC) of the coumarin derivatives in model bacterial strains. The x-axis compounds 1-5 used sequentially. The y-axis shows the MIC value in $\mu g/mL^{-1}$

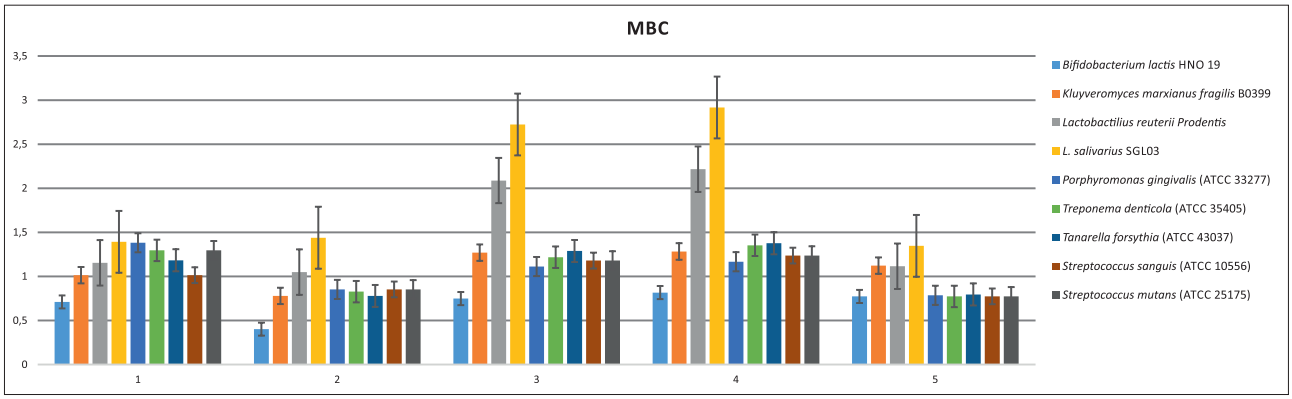

Fig. 4. Minimum bactericidal concentration (MBC) of coumarin derivatives in model bacterial strains. The x-axis features compounds 1-5 used sequentially. The y-axis shows the MBC value in $\mu g/mL^{-1}$

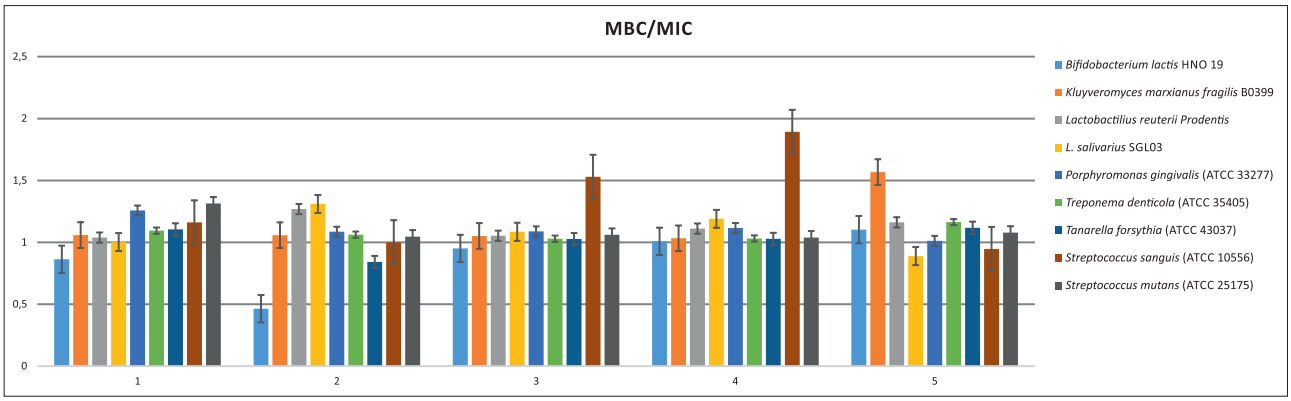

Fig. 5. The ratio of MBC/MIC of the investigated agents 1-16 in selected bacterial strains. The x-axis compounds 1-5. The y-axis shows the MBC/MIC value in μM shows the MIC value in μM

to achieve drug concentrations that eradicate 99.9% of the exposed organisms. Should the proportion of antibiotic be classified as bacteriostatic; nevertheless, the MBC to MIC ratio is high and it may not be safe to take doses of the medication to kill 99.9% of bacteria. The exact distinction between the bacteriostatic and bactericidal properties of many substances depends on the concentration of the pathogen and the drug that is reached in the target tissue. In the case of all tested compounds 1-5, we are dealing with bactericidal agents (fig. 5).

Furthermore, as can be seen in figures 3 and 5, investigated compounds typically exhibited greater antibacterial activity than commonly used antibiotics. This is especially crucial because tested microorganism resistance to known antibiotics has evidently increased, such as cloxacillin (clox) or ciprofloxacin (cipro) (fig. 6). The rate at which microorganisms will become resistant to bleomycin (bleo) is unknown, but this will cause a major disruption in the antibiotic arsenal that is now used to treat hospital infections. Interestingly, our compounds exhibit activity comparable to that of bleomycin (bleo).

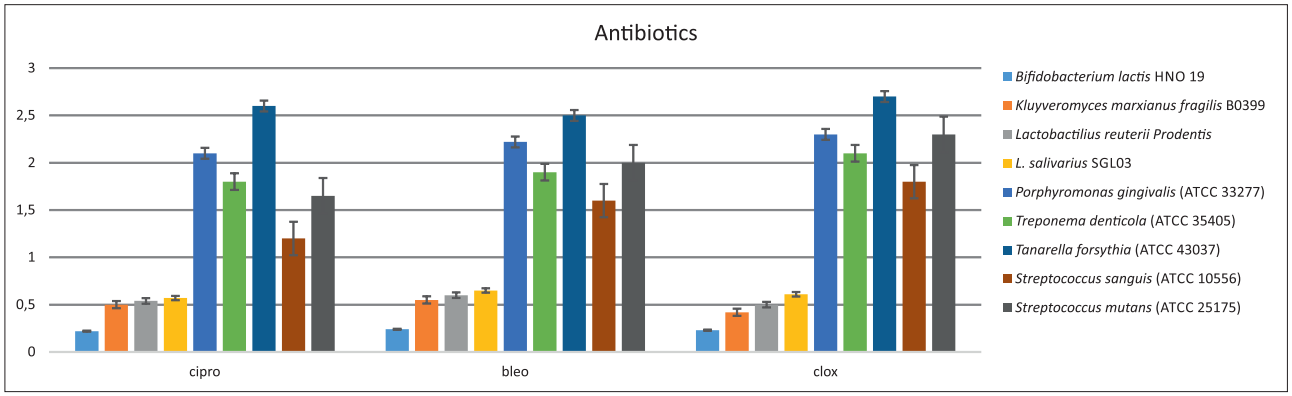

Fig. 6. Examples of MIC in selected bacterial strains for studied antibiotics ciprofloxacin (cipro), bleomycin (bleo), and cloxacillin (clox). The x-axis features antibiotics used sequentially. The y-axis shows the MIC value in µM

Tab. 1. Statistical analysis of all analyzed compounds by MIC, MBC, and MBC/MIC; * $p < 0.05$,** $p < 0.01$, and *** $p < 0.001$

No. of samples	1, 2	3	4-5	Type of test
Bifidobacterium lactis HNO 19	*	**	**	MIC
Kluyveromyces marxianus fragilis B0399	*	**	**	MIC
Lactobactilius reuterii Prodentis	*	**	**	MIC
Lactobacillus salivarius SGL03	*	**	**	MIC
Porphyromonas gingivalis (ATCC 33277)	*	**	***	MBC
Treponema denticola (ATCC 35405)	*	**	***	MBC
Tanarella forsythia (ATCC 43037)	*	**	***	MBC
Streptococcus sanguis (ATCC 10556)	*	**	***	MBC
Streptococcus mutans (ATCC 25175)	*	**	*	MIC

Model of analysed strains with tested compounds were statis-tical significance at $p < 0.05$ (tab. 1).

Discussion

Cytotoxic studies of tested compounds

The effects of the 5 compounds investigated (illustrated in fig. 3-5) were evaluated within bacterial cells using a previously established methodology (21, 47). Following the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), the MIC values ranged between 0.25-4.5 µM, while the MBC values fell within the range of 1-8 (+/- 0.5) µM for bacterial strains of red and yellow complexes: *Porphyromonas gingivalis* (ATCC 33277), *Treponema denticola* (ATCC 35405), *Tanarella forsythia* (ATCC 43037), *Streptococcus sanguis* (ATCC 10556), *Streptococcus mutans* (ATCC 25175) and as a probiotic strains: *Bifidobacterium lactis* HNO 19, *Kluyveromyces marxianus fragilis* B0399, *Lactobactilius reuterii Prodentis*, *Lactobacillus salivarius* SGL03.

MIC serves as a reference for assessing the susceptibility or resistance of bacterial strains to the antibiotic applied in vitro. On the contrary, MBC represents the minimum concentration of an antibacterial agent required to eradicate bacteria, distinguishing it as bactericidal rather than bacteriostatic (47). Basically, all our compounds tested against Gram-positive and Gram-negative bacterial strains show good activity against them. And all MIC values are below 5 µg/mL^{-1} (fig. 3). Interestingly, the change in the different toothpaste and gels had a vital impact on activity. The oral hygiene products containing active ingredients in their composition was playing a role in the activity against some bacteria, which we confirmed with the activity of compound no. 1 and 5. This means that the active components had a good potency compared to antibiotics (fig. 6). Interestingly, all compounds showing broad spectrum activity against both Gram negative as well as gram positive bacteria but the highest the compound no 3 and 4. Compounds 1 to 5 have greater efficacy against *Porphyromonas gingivalis* (ATCC 33277), *Treponema denticola* (ATCC 35405), *Tanarella forsythia* (ATCC 43037), *Streptococcus sanguis* (ATCC 10556), but showed also moderate efficacy against *Streptococcus mutans* (ATCC 25175) strains. Compounds No. 3 and 4 demonstrated a broad range and good action against all bacteria, as revealed confidentially with respect to the fluorine group. Furthermore, compounds with a parafluoro group in had more activity overall. The obtained results were a good and very simple and cheap training test which allowed us to estimate to what extent the types of specific diets have an

influence on the formation of specific bacterial biofilms on the induction of inflammatory conditions of periodontal disease with specific bacterial biofilms. The methods described in the manuscript significantly shorten the time of detection of disease entities induced by persistent bacterial biofilms belonging to different classes. They also allow the assessment of the actual inflammation of the periodontal tissues. By specifying the type of identifier, the doctor can directly estimate what type of bacteria he is dealing with and what treatment should be administered. The obtained results suggest that the substances contained in the analysed components may also interact with red complexes of bacteria showing cariogenic activity present in the human oral cavity (11, 12). Currently, antibiotic resistance among pathogenic bacteria is becoming more and more common, leading to super-resistance. The lack of colonization capabilities by such pathogens is the result of the lack of access to adhesins occupied by the microflora of the inhabited niche. Moreover, the sessile microflora can produce certain antagonistic compounds that interfere with the colonization of the niche by exogenous bacterial strains, e.g. hydrogen peroxide produced by S. mitis, salivaricin produced by *S. salivarius* streptococci (which inhibits the development of pyogenic streptococci *S. pyogenes*) or enocin (bacteriocin) produced by *S. mutans*, C. martuchotii and A. actinomycetemcomitans (which inhibits the growth of related organisms) of yellow and red complexes (36).

Conclusions

The observed results are especially important because of increasing resistance of bacteria to various drugs and antibiotics. All selected compounds showed super-selectivity in all analyzed bacterial strains and exhibited the highest cytotoxic activity, comparable or better than the commonly used antibiotics: ciprofloxacin, bleomycin, and cloxacillin. The bacterial flora of the oral cavity contributes to the development or aggravation of oxidative stress. As mentioned above, bacteria inhabiting the crowns of teeth breathe oxygen (Eh +200 mV) and produce carbon dioxide, which results in a decrease in the oxidative reduction potential to about +76 mV. As the bacterial plaque grows, this potential can decrease to as much as -140 mV. In the gingival crevices and pockets – in the subgingival dental plaque, the oxidative reduction potential fluctuates in the range of Eh +72 mV to -300 mV, which leads to the creation of an anaerobic environment. People with high levels of oxidative stress suffer from periodontal diseases twice as often and vice versa – periodontal diseases closely correlate with an increase in the level of oxidative stress parameters (22-26). The analyzed probiotic strains were resistant to the action of pastes and gels, while the pathogenic strains were destroyed by strong cytotoxic properties damaging both gram-negative and gram-positive pathogens. This indicates their selective action on red and yellow complex bacteria.

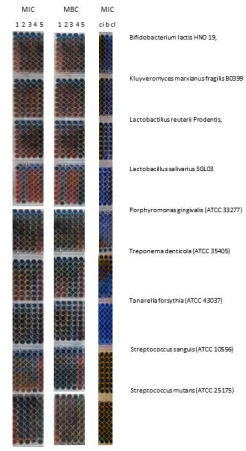

Fig. S1. Examples of MIC on microplates with different concentration of studied compounds (μg/mL^{-1}). Resazurin was added as an indicator of microbial growth with *Porphyromonas gingivalis* (ATCC 33277), *Treponema denticola* (ATCC 35405), *Tanarella forsythia* (ATCC 43037), *Streptococcus sanguis* (ATCC 10556), *Streptococcus mutans* (ATCC 25175) and as a probiotic strains; *Bifidobacterium lactis* HNO 19, *Kluyveromyces marxianus fragilis* B0399, *Lactobactilius reuterii Prodentis*, *Lactobacillus salivarius* SGL03, additionally, examples of MIC with different studied antibiotics with ciprofloxacin (ci), bleomycin (b), and cloxacillin (cl) in (μg/mL^{-1})

Conflict of interest
Konflikt interesów

None
Brak konfliktu interesów

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References/Piśmiennictwo

1. Pietruska M, Dolińska E, Skurska-Czelej A: Zapobieganie chorobom przyzębia i ich leczenie. Wydawnictwo Czelej, Lublin 2018.
2. Harsman P (red. wyd. pol. U. Kaczmarek): Stomatologia zachowawcza, stomatologia dziecięca, ortodoncja, periodontologia, protetyka. Elsevier Urban & Partner, Wrocław 2010.
3. Coulthard P, Horner K, Sloan P, Theaker E: Choroby błony śluzowej jamy ustnej, radiologia, chirurgia stomatologiczna. Elsevier Urban & Partner, Wrocław 2008.
4. Górska R (red.): Diagnostyka i leczenie chorób błony śluzowej jamy ustnej. Wydawnictwo Med Tur Press International, Otwock 2011.
5. Jańczuk Z (red.): Praktyczna periodontologia kliniczna. Wydawnictwo Kwintesencja, Warszawa 2004.
6. Jańczuk Z (red.): Profilaktyka profesjonalna w stomatologii. Wydawnictwo Lekarskie PZWL, Warszawa 2001.

7. Felton A, Chapman A, Felton S (red. wyd. pol. U. Kaczmarek): Zdrowie jamy ustnej. Wydawnictwo Lekarskie PZWL, Warszawa 2009.
8. Jańczuk Z (red.): Podręcznik dla asystentek i higienistek stomatologicznych. Wydawnictwo Lekarskie PZWL, Warszawa 2011.
9. Mielczarek A, Kowalik R, Najman M (red.): Podręcznik dla asystentek i higienistek stomatologicznych. Wydawnictwo Lekarskie PZWL, Warszawa 2018.
10. Krajewski W: Podstawy profilaktyki stomatologicznej. Wydawnictwo Med Tour Press International, Otwock 2019.
11. Lewis MAO, Jordan RCK Medycyna jamy ustnej. Wydawnictwo Lekarskie PZWL, Warszawa 2013.
12. Borakowska-Siennicka M: Wpływ diety na stan tkanek przyzębia – na podstawie piśmiennictwa. Nowa Stomatol 2012; 17 (3): 130-133.
13. Górska R: Choroby przyzębia. Klasyfikacja 2017. Wydawnictwo Lekarskie PZWL, Warszawa 2018.
14. Konopka T, Gerber H: Stan kliniczny przyzębia pacjentów badanych w kierunku stresu oksydacyjnego związanego z zapaleniem przyzębia, Dent. Med. Probl 2008; 45(1): 42-49.
15. Rutkowski R, Pancewicz SA, Rutkowski K, Rutkowska J: Znaczenie reaktywnych form tlenu i azotu w patomechanizmie procesu zapalnego. Pol Merkuriusz Lek 2007; 23(134): 131-136.
16. Kamecka-Białowarczuk EA, Dąbrowska E: Równowaga oksydoredukcyjna w środowisku jamy ustnej. e-Dentico 2009; 2: 58-68.
17. Kamecka-Białowarczuk EA, Dąbrowska E: Równowaga oksydoredukcyjna w środowisku jamy ustnej. e-Dentico 2008; 2: 42-51.
18. Łuszczewski A, Matyska-Piekarska E, Trefler J et al.: Reaktywne formy tlenu – znaczenie w fizjologii i stanach patologii organizmu; Reumatologia 2007; 45(5): 284-289.
19. Olędzki R, Kędziora-Kornatowska K: Mechanizmy antyoksydacyjne w organizmie człowieka. Valetudinaria – Post Med Klin Wojsk 2006; 11(1): 15-20.
20. Król K, Grocholewicz K: Wybrane białka śliny jako biomarkery miejscowych i ogólnych procesów chorobowych – przegląd piśmiennictwa. Zakład Stomatologii Ogólnej Pomorskiej Akademii w Szczecinie 2007; 53(1): 78-82.
21. Kucia M, Wietrak E, Szymczak M, Kowalczyk P: Preliminary in vitro study on effect of *Lactobacillus salivarius* and other natural components against anaerobic periodontal bacteria. Molecules 2020; 25: 4519.
22. Caton JG, Armitage G, Berglundh T et al.: New classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. J Clin Periodontol 2018; 45 (suppl. S20): S1-S8.
23. Chen Y, Yang YC, Zhu BL et al.: Association between periodontal disease, tooth loss and liver diseases risk. J Clin Periodontol 2020; 47(9): 1053-1063.
24. Carrizales-Sepúlveda EF, Ordaz-Farías A, Vera-Pineda R, Flores-Ramírez R: Periodontal Disease, Systemic Inflammation and the Risk of Cardiovascular Disease. Heart Lung Circ 2018; 27(11): 1327-1334.
25. Sroussi HY, Epstein JB, Bensadoun RJ et al.: Common oral complications of head and neck cancer radiation therapy: mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis. Cancer Med 2017; 6(12): 2918-2931.
26. Güll FD, Deppe H, Kesting M, Schwarzer C: Periodontal disease-like bone loss after adjuvant radiotherapy in the head and neck region: A case report and review of the literature. Quintessence Int 2017; 48(6): 451-457.
27. Rowińska I, Szyperska-Ślaska A, Zariczny P et al.: Impact of the Diet on the Formation of Oxidative Stress and Inflammation Induced by Bacterial Biofilm in the Oral Cavity. Materials 2021; 14: 1372.
28. Rowińska I, Szyperska-Ślaska A, Zariczny P et al.: The Influence of Diet on Oxidative Stress and Inflammation Induced by Bacterial Biofilms in the Human Oral Cavity. Materials 2021; 14: 1444.
29. Smith AJ, Hall V, Thakker B, Gemmell CG: Antimicrobial susceptibility testing of Actinomyces species with 12 antimicrobial agents. J Antimicrob Chemother 2005; 56: 407-409.

30. Hansen JM, Fjeldsoe-Nielsen H, Sulim A et al.: Actinomyces species: A Danish survey on human infections and microbial characteristics. Open Microbiol J 2009; 3: 113.
31. Brook I: Actinomycosis: diagnosis and management. South Med J 2008; 101: 1019-1023.
32. Hall V: Actinomyces gathering evidence of human colonization and infection. Anaerobe 2008; 14: 1-7.
33. Binda C, Lopetuso LR, Rizzatti G et al.: Actinobacteria: A relevant minority for the maintenance of gut homeostasis. Dig Liver Dis 2018; 50(5): 421-428.
34. Socransky SS, Haffajee AD, Cugini MA et al.: Microbial complexes in subgingival plaque. J Clin Periodontol 1998; 25: 134-144.
35. Chaves BD, Brashears MM, Nightingale KK: Applications and safety considerations of *Lactobacillus salivarius* as a probiotic in animal and human health. J Appl Microbiol 2017; 123: 18-28.
36. Darveau RP, Hajishengallis G, Curtis MA: *Porphyromonas gingivalis* as a potential community activist for disease. J Dent Res 2012; 91: 816-820.
37. Jayaram P, Chatterjee A, Raghunathan V: Probiotics in the treatment of periodontal disease: A systematic review. J Indian Soc Periodontol 2016; 20: 488-495.
38. Kadowaki T, Baba A, Abe N et al.: Suppression of pathogenicity of *Porphyromonas gingivalis* by newly developed gingipain inhibitors. Mol Pharmacol 2004; 66: 1599-1606.
39. Haffajee AD, Socransky SS, Patel MR, Song X: Microbial complexes in supragingival plaque. Oral Microbiol Immunol 2008; 23: 196-205.
40. Nędzi-Góra M, Kowalski J, Krajewski J, Górska R: Microbiological analysis of deep periodontal pockets in people with chronic periodontitis by PCR. Stomatol J 2007; 11: 717-725.
41. Paik S, Senty L, Das S et al.: Identification of Virulence Determinants for Endocarditis in Streptococcus sanguinis by Signature-Tagged Mutagenesis. Infect Immun 2005; 73: 6064-6074.
42. Servin AL: Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol Rev 2004; 28: 405-440.
43. Sela MN: Role of *Treponema denticola* in periodontal diseases. Crit Rev Oral Biol Med 2001; 12: 399-413.
44. Cutler CW, Kalmar JR, Genco CA: Pathogenic strategies of the oral anaerobe *Porphyromonas gingivalis*. Trends Microbiol 1995; 3: 45-51.
45. Neville BA, O'Toole PW: Probiotic properties of *Lactobacillus salivarius* and closely related Lactobacillus species. Future Microbiol 2010; 5: 759-774.
46. Pidutti P, Federici F, Brandi J et al.: Purification and characterization of ribosomal proteins L27 and L30 having antimicrobial activity produced by the *Lactobacillus salivarius* SGL 03. J Appl Microbiol 2018; 124: 398-407.
47. Kowalczyk P, Koszelewski D, Brodzka A et al.: Evaluation of Antibacterial Activity against Nosocomial Pathogens of an Enzymatically Derived α-Aminophosphonates Possessing Coumarin Scaffold. Int J Mol Sci 2023; 24: 14886.

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