Biofilm-forming Ability of Anaerobic Bacterial Strains Isolated from Patients Diagnosed with Periodontitis

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Abstract: Bacterial biofilms represent the primary causative agents of pathogenic processes within the oral cavity. Biofilm microorganisms exhibit heightened resistance to adverse environmental factors. The objective of this study was to ascertain the biofilm-forming potential of clinical strains of anaerobic microorganisms isolated from the periodontal pocket of patients diagnosed with periodontitis. The study of biofilm formation was carried out by the method (Djordjevic et al., 2002) using flat-bottomed 96-well polystyrene microtitration plates. Biofilm formation was determined by staining with 0.1% crystal violet. The study utilised clinical strains isolated from the periodontal pocket of 61 patients diagnosed with grade III chronic periodontitis. Of these, 30 strains belonging to three bacterial species were selected for further analysis: *Porphyromonas asaccharolytica*, *Slackia exigua*, and *Schaalia odontolytica*. Among the *Porphyromonas asaccharolytica* strains, 80% formed biofilm; the figure for *Slackia exigua* was 90%, and for *Schaalia odontolytica* – 80%. In total, 25 strains were capable of biofilm formation. The results indicate that the isolated strains of *Porphyromonas asaccharolytica*, *Slackia exigua*, and *Schaalia odontolytica* exhibited a significant capacity for biofilm formation (83.3% of the strains formed biofilm), particularly *Slackia exigua* strains, which exhibited the highest number of strains with high biofilm formation ability – 5 (16.6%). It is also noteworthy that these microorganisms exhibited a moderate persistence frequency (16.4% each), of the total sample of microbial biocenoses from inflammatory periodontal soft tissue areas.

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Introduction

Bacterial biofilms represent the primary causative agents of pathogenic processes within the oral cavity (Marsh and Devine, 2011). These biofilms form on both the surface of the oral mucosa and synthetic materials, resulting in the development of persistent inflammatory processes and a substantial reduction in the service life of dental prostheses and restorative materials (Auschill et al., 2002). Oral biofilms have the capacity to affect the tissues surrounding the tooth, causing gingival inflammation and, if persistent, damage to the alveolar ridge, which can ultimately result in tooth loss (Papapanou et al., 2018).

Biofilms are defined as conglomerates of microorganisms (bacteria, algae and fungi) that attach to biological and non-biological surfaces and are functionally organised in layers (Marsh, 2005). Biofilm microorganisms exhibit enhanced resistance to adverse environmental factors (Chia et al., 2008). The life processes within a biofilm differ significantly from those observed in the planktonic state (Marsh, 2004; Shemesh et al., 2007). In addition to mechanical stability, the formation of biofilms stimulates synergistic interactions, ensures survival during periods of starvation and exposure to antimicrobial agents, and prevents the displacement of extracellular enzymes (Tzu et al., 2004). In this sense, biofilms are not merely conglomerates of microorganisms, but rather well-organised matrix systems with a clear distribution of functions. Individual prokaryotes "communicate" within the system by transmitting signals that modulate gene expression. In addition to this, biofilms perform various functions in order to ensure their own survival (Brown et al., 2005; Shemesh et al., 2010). One of the main functions of biofilms is the secretion of extracellular polymeric substances (EPS) to protect microorganisms from external influences (Flemming and Wingender, 2010).

Okahashi et al. (2011) demonstrated that the predominant primary colonisers of teeth are gram-positive facultative anaerobic cocci and bacilli, including *Streptococcus* and *Actinomyces* species. These bacteria provide the basis for the further development of dental biofilm. After these microorganisms attach to the surface, a mass of biofilm develops through continuous growth and further adsorption of other bacterial species by coagulation. The biofilm-forming microorganisms secrete enzymes and toxins that stimulate the human body to synthesise antibodies and cytokines, which are retained by the biofilm matrix, exacerbating the inflammatory process (Leonteva et al., 2023).

The surface molecules of these primary colonisers allow for the coaggregation of gram-negative bacteria that have a lower level of adhesion to the pelvis, including members of the *Veillonella* and *Fusobacterium* genera. Bacteria belonging to the *Fusobacterium* genus, such as *Fusobacterium nucleatum*, are capable of co-aggregating with both primary and secondary colonisers, hence their name "bridge bacteria", and they are known to contribute to the successful development of dental biofilm. The mechanism by which *F. nucleatum* aggregates with neighbouring bacteria involves surface molecules such as RadD, arginine-inhibitory adhesin, and fusobacterial apoptosis protein Fap2 (Kaplan et al., 2010).

Another study (Mark Welch et al., 2016) utilised a combination of labelling and spectral imaging FISH (CLASI-FISH) to demonstrate that the composition of biofilms was characterised by a complex microbial consortium, termed the "hedgehog structure". This structure consisted primarily of nine taxa arranged in an organised spatial structure, including *Corynebacterium*, *Streptococcus*, *Porphyromonas*, *Haemophilus/Aggregatibacter*, *Neisseriaceae*, *Fusobacterium*, *Leptotrichia*, *Capnocytophaga*, and *Actinomyces*.

It is also known that bacteria in the biofilm exhibit a higher degree of resistance to antibiotics and antimicrobial agents, so the ability to form biofilms is considered one of the factors of pathogenicity (Shahzad et al., 2015). The formation of multispecies biofilms, which include periodontopathogenic microorganisms, has been demonstrated to cause the progression of periodontal diseases through microbial interactions (Meyle and Chapple, 2015; Sanz et al., 2017). Biofilms on the teeth surface, especially on porous hydroxyapatite, have been shown to contribute to the progression of periodontitis by facilitating bacterial attachment and growth (Jaffar et al., 2016).

Objective

The objective of this study was to ascertain the biofilm-forming activity of clinical strains of anaerobic microorganisms isolated from the periodontal pocket of patients diagnosed with periodontitis.

Material and Methods

Strains and cultivation conditions

For the purposes of this study, clinical strains were isolated from the periodontal pocket of 61 patients diagnosed with grade III chronic periodontitis at the Dental Outpatient Clinic of the Dental Faculty of the Uzhhorod National University. The isolation of pure cultures was carried out by the sector sowing method according to Gold using Schaedler agar + 5%

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defibrinated sheep blood (Condalab, Spain) nutrient medium containing (on a g/l basis): bacteriological agar – 13.5; hemin – 0.01 g/l; peptone mixture – 5; trypto-casein soy broth – 10; dextrose – 5; L-cysteine – 0.4; yeast extract – 5; Tris (hydroxymethyl aminomethane) – 3.To create anaerobic conditions, an anaerobic container with an anaerobic system (AnaeroGen System – "Oxoid", UK) was used. The pure cultures were grown in Schaedler broth (Condalab, Spain) containing (on a g/l basis): casein peptone – 2.5; hemin – 0.01; meat peptone – 2.5; trypto-casein soy broth – 10; dextrose – 5; L-cysteine – 0.4; yeast extract – 5; Tris (hydroxymethyl aminomethane) – 3. The cultures were then subjected to incubation under anaerobic conditions at 37 °C for a period of 48 hours. The identification of the isolated pure cultures was conducted through the utilisation of MALDI-TOF (matrix-assisted laser desorption/ ionisation and time-of-flight mass spectrometry).

Biofilm analysis

The study of biofilm formation was carried out by the method (Djordjevic et al., 2002) using flat-bottomed 96-well polystyrene microtitration plates (Starlab, Ukraine). Prior to plating, the 48-hour cultures of periodontal pathogens were diluted in fresh sterile Schaedler broth to an optical density (OD) of 0.05 at 600 nm. The inoculum was then injected into the wells using a Thermo Scientific F1 multichannel variable volume pipette (Thermo Fisher Scientific, USA), with 200 µl aliquots per well. Sterile Schaedler broth with the same volume was used as a negative control. The inoculated plates were then incubated under stable anaerobic conditions at 37 °C for 72 hours, after which biofilm formation was periodically assessed by visual inspection.

Quantification of biofilm formation

Biofilm formation was determined by staining with 0.1% crystal violet, as described above (Pratt and Kolter, 1998). The microtiter plate containing the biofilm was gently washed several times with distilled water, using sterile Pasteur pipettes (Starlab, Ukraine). The plates were then dried by tapping them on a paper towel. This cleaning procedure was undertaken to ensure the removal of any loosely attached cells or broth residues that might otherwise be stained during the subsequent step. To determine the total biofilm mass, 200 µl of 0.1% crystal violet was added to each well for 30 minutes (Sol et al., 2013). The plate was then gently rinsed with distilled water and allowed to air-dry in an incubator at 37 °C for 15 minutes. The remaining biofilm was then visualised using photography. Following this, the stained biofilm was dissolved for 30 minutes by adding 200 µl of 95% ethanol to each well. The plates were then read using a semi-automatic ELISA analyser with a photometric system for microplates RT-6100 (Rayto, China) at an absorption of 492 nm (Stepanović et al., 2007).

Statistical analysis

The data from a minimum of three independent experiments are presented as mean ± SD (standard deviation). Comparisons were performed by Student's *t*-test using Microsoft Office Excel 2016 software.

Figure 1: Pure culture of Slackia exigua on Schaedler agar + 5% defibrinated sheep blood.

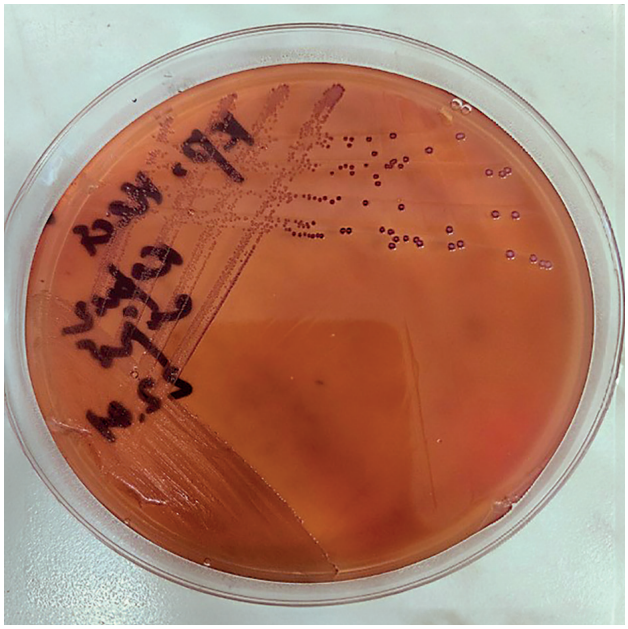

Figure 2: Pure culture of Schaalia odontolytica on Schaedler agar + 5% defibrinated sheep blood.

Results

The present study investigates the formation of biofilm *in vitro* in strains of obligate anaerobes isolated from the inflammatory process in the periodontal pocket of patients diagnosed with periodontitis. A total of 61 patients with grade III chronic periodontitis were included in the study, and material was taken from their periodontal pockets. Of these, 30 bacterial strains belonging to 3 species were selected for further study: *Porphyromonas asaccharolytica*, *Slackia exigua*, and *Schaalia odontolytica* (Figures 1–3). The overall persistence rate of these microorganisms was 16.4% across the entire sample.

In the study, the formation of biofilm was observed in 80% of *Porphyromonas asaccharolytica* strains, 90% of *Slackia exigua* strains, and 80% of *Schaalia odontolytica* strains. The investigation revealed that a total of 25 strains were capable of biofilm formation, as illustrated in Figure 4.

The microtitration plate shown in Figure 5 presents an *in vitro* biofilm from the strains of obligate anaerobes, with the optical density of different strains

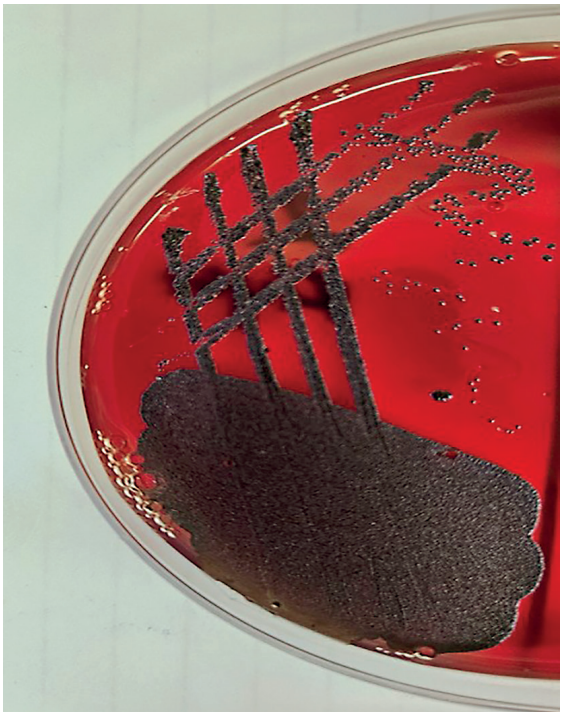

Figure 3: Pure culture of Porphyromonas asaccharolytica on Schaedler agar + 5% defibrinated sheep blood.

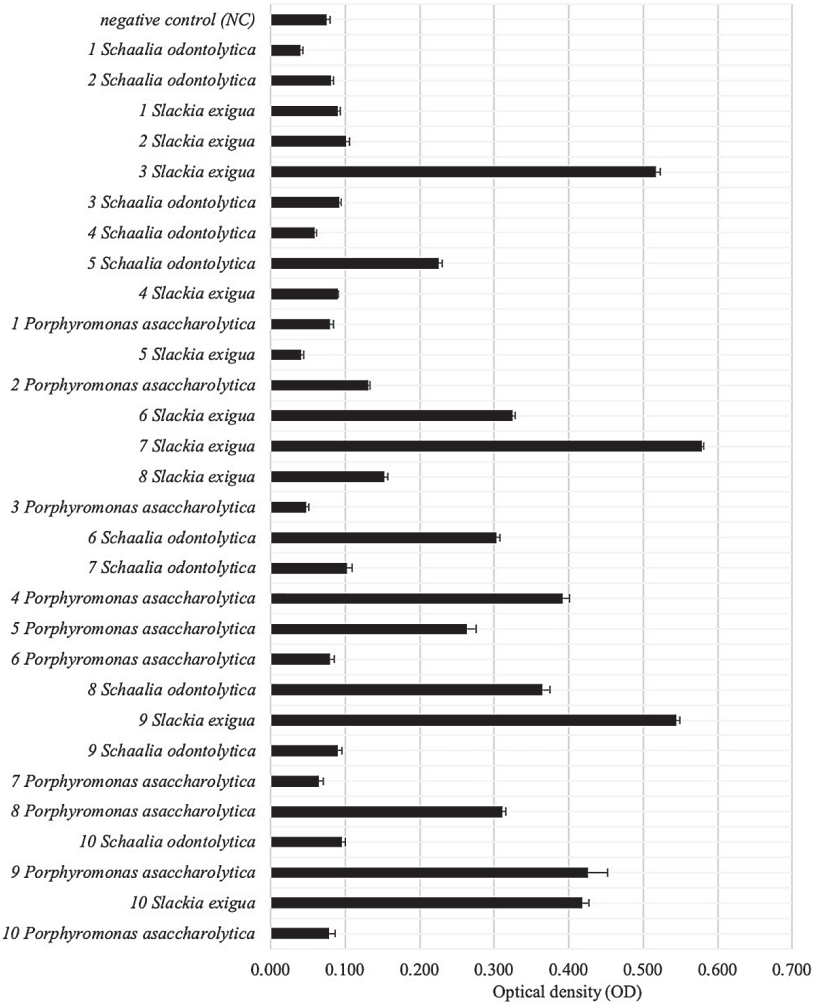

Figure 4: Biofilm indicators of Porphyromonas asaccharolytica, Slackia exigua, and Schaalia odontolytica strains. Sterile Schaedler broth was utilised as a negative control (NC), with a value of 0.075. The mean value is represented by wide bars, whilst the standard deviation (SD) is indicated by error bars.

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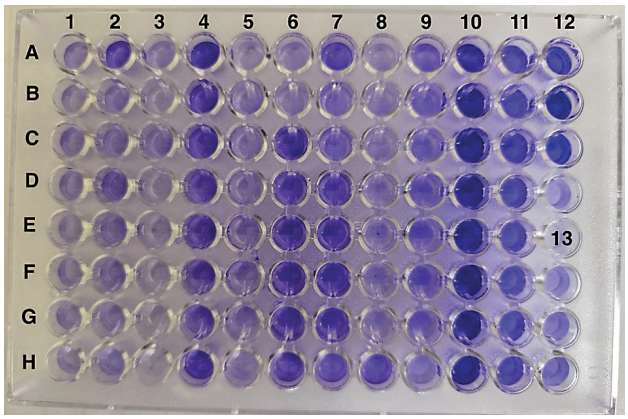

Figure 5: A microtitration plate with biofilm in vitro in strains of obligate anaerobes: **1** *– 9 Schaalia odontolytica;* **2** *– 2 Slackia exigua;* **3** *– 10 Porphyromonas asaccharolytica;* **4** *– 6 Schaalia odontolytica;* **5** *– 7 Schaalia odontolytica;* **6** *– 6 Slackia exigua;* **7** *– 8 Schaalia odontolytica;* **8** *– 10 Schaalia odontolytica;* **9** *– 8 Slackia exigua;* **10** *– 7 Slackia exigua;* **11** *– 8 Porphyromonas asaccharolytica;* **12 (A–D)** *– 9 Porphyromonas asaccharolytica;* **13 (E–H)** *– 2 Porphyromonas asaccharolytica.*

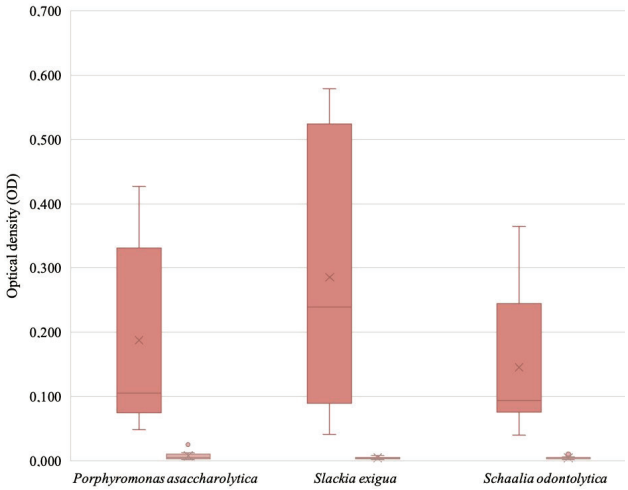

Figure 6: The biofilm formation ability of anaerobic bacteria isolated from the periodontal pocket. Note: The median is indicated by a horizontal line within each block. The error bars represent the minimum and maximum values, and the spread between quartiles represents the variability of the results.

depending on the intensity of staining with 0.1% crystal violet clearly demonstrated.

The following classification system was utilised to interpret the biofilm formation by strains: non-biofilm-forming, low biofilm forming, moderate and high biofilm forming (see Tables 1 and 2 for the data).

OD (strain) ≤ OD (control) = no biofilm formation;
OD (control) ≤ OD (strain) ≤ 2OD (control) = low biofilm formation;
2OD (control) ≤ OD (strain) ≤ 4OD (control) = moderate biofilm formation;
4OD (control) ≤ OD (strain) = high biofilm formation (Stepanović et al., 2007).

A comparative analysis of the optical density of the strains of each species (Figure 6) reveals that *Slackia exigua* strains exhibit the most extensive data scatter and the highest median. This finding suggests that these strains possess the most pronounced biofilm formation ability (within the experimental parameters), with their indicators demonstrating significant variability. In contrast, *Porphyromonas asaccharolytica* exhibited a comparatively diminished data scatter

Table 1: Formation of biofilms by anaerobic periodontal pathogens, distribution by classification (n=30)

Microorganisms	No biofilm formation	Number of biofilm-forming strains		
		low	moderate	high
Porphyromonas asaccharolytica	2	4	1	3
Slackia exigua	1	3	1	5
Schaalia odontolytica	2	5	1	2

Table 2: Optical density of biofilms by anaerobic periodontal pathogens, distribution by classification (n=30)

Microorganisms	Biofilm forming density, in OD		
	low	moderate	high
Porphyromonas asaccharolytica	0.105 ± 0.027	0.263	0.367 ± 0.060
Slackia exigua	0.095 ± 0.006	0.153	0.450 ± 0.145
Schaalia odontolytica	0.092 ± 0.011	0.226	0.334 ± 0.031

OD – optical density

and median, indicative of a less pronounced biofilm-forming capacity that is more stable than that observed in *Slackia exigua*. *Schaalia odontolytica* demonstrated characteristics analogous to those of *Porphyromonas asaccharolytica*, yet with a marginally lower median and a more extensive data scatter. This suggests that while these strains do possess biofilm-forming capabilities, the extent and variability of their responses may vary.

Discussion

The results obtained demonstrate the role of the studied species in the formation of inflammatory periodontal diseases. *S. exigua* has been frequently isolated from periradicular lesions (Sato et al., 1993; Kiryu et al., 1994; Hashimura et al., 2001), as well as from other areas of the oral cavity (Moore et al., 1983; Hill et al., 1987; Wade et al., 1994). In addition, *S. exigua* has been reported to be associated with clinical indicators of periodontal disease (Booth et al., 2004). In one study, two cases of monomicrobial bacteraemia caused by *S. exigua* isolated from two healthcare facilities were reported (Kawasuji et al., 2020); a case of systemic infection caused by obligate anaerobes was also reported: *Dialister pneumosintes* and *Slackia exigua*, arising from an acute periapical abscess (Lee et al., 2016). Bukki et al. (2011) reported the first case of *S. exigua* isolation from cerebrospinal fluid in a patient with suspected enterospinal fistula and subsequent polymicrobial exudative meningitis. A further case of hip prosthesis infection associated with anaerobic gram-positive *Slackia exigua* was also described by Rieber et al. (2019). This was isolated from periprosthetic tissues and prosthesis components after invasive dental procedures, and the patient had not received antibiotic prophylaxis before the dental intervention. A rare instance of bacteremia caused by *Porphyromonas asaccharolytica* in a patient diagnosed with necrotising fasciitis was documented by Cobo et al. (2021). Gupta et al. (2018) delineated the case of a patient with Lemierre's syndrome caused by obligate anaerobe *Porphyromonas asaccharolytica* from the oral cavity. Stašková et al. (2021) investigated the antibiofilm activity of neutralised cell-free supernant (nCFS) of *Streptococcus salivarius* K12 at different concentrations against *Schaalia odontolytica* P10, showing a significant reduction ($p<0.001$) in biofilm formation at nCFS concentrations of 60 and 30 mg/ml (37). In the study conducted by Heller et al. (2016), early dental biofilm samples were analysed from 11 healthy individuals. The semi-quantitative HOMIM method was utilised, resulting in the identification of at least 92 species. Among these, *Slackia exigua* and other species demonstrated a high persistence frequency.

As demonstrated in our previous studies, opportunistic pathogens that persist in the context of chronic inflammation of periodontal tissues have the capacity to form a biofilm (Kryvtsova and Kostenko, 2020). Furthermore, there is evidence to suggest that bacteria of the *Staphylococcus* genus possess genetic determinants associated with biofilm formation. Consequently, determining the bacteria's capacity to form a biofilm is imperative in order to develop a treatment strategy that utilises substances with antibiofilm-forming properties. The capacity of *Porphyromonas asaccharolytica*, *Slackia exigua*, and *Schaalia odontolytica* bacteria isolated from periodontal pockets of patients diagnosed with periodontitis to form biofilms underscores their role in the development of inflammatory periodontal diseases.

Conclusion

The results of this study demonstrate that the isolated strains of *Porphyromonas asaccharolytica*, *Slackia exigua*, and *Schaalia odontolytica* exhibited a notable capacity to form biofilms (83.3% of strains formed biofilms), particularly *Slackia exigua* strains, which exhibited the highest number of strains with high biofilm formation ability – 5 (16.6%). It is also noteworthy that these microorganisms exhibited a moderate frequency of persistence, with 16.4% of the total sample of microbial biocenoses of inflammatory periodontal soft tissues being represented by each. The results of the study suggest the potential for further research and the establishment of the role of these microorganisms in periodontal inflammatory diseases.

References

Auschill, T., Arweiler, N., Brecx, M., Reich, E., Sculean, A., Netuschil, L. (2002) The effect of dental restorative materials on dental biofilm. *Eur. J. Oral Sci.* **110(1)**, 48–53.

Booth, V., Downes, J., Van den Berg, J., Wade, W. G. (2004) Gram-positive anaerobic bacilli in human periodontal disease. *J. Periodontal Res.* **39(3)**, 213–220.

Brown, T., Ahn, S., Frank, R., Chen, Y., Lemos, J., Burne, R. (2005) A hypothetical protein of *Streptococcus mutans* is critical for biofilm formation. *Infect. Immun*. **73(5)**, 3147–3151.

Bukki, J., Huttner, H. B., Lee, D. H., Jantsch, J., Janka, R., Ostgathe, C. (2011) Polymicrobial feculent meningitis with detection of *Slackia exigua* in the cerebrospinal fluid of a patient with advanced rectal carcinoma. *J. Clin. Oncol.* **29**, 852–854.

Chia, N., Woese, C., Goldenfeld, N. (2008) A collective mechanism for phase variation in biofilms. *Proc. Natl. Acad. Sci. U. S. A.* **105(38)**, 14597–14602.

Cobo, F., Pérez-Carrasco, V., Sánchez-Martin, V., García-Salcedo, J.

A., Barrón Martín, E., Navarro-Marí, J. M. (2021) A rare cause of bacteremia due to *Porphyromonas asaccharolytica* in a patient with necrotizing fasciitis. *Anaerobe* **71**, *102442*.

Djordjevic, D., Wiedmann, M., McLandsborough, L. A. (2002) Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. *Appl. Environ. Microbiol.* **68(6)**, 2950–2958.

Flemming, H., Wingender, J. (2010) The biofilm matrix. *Nat. Rev. Microbiol*. **8(9)**, 623–633.

Gupta, M., Annam, R., Bahgat, J., Eng, M. (2018) *Porphyromonas asaccharolytica* as a rare causative agent for Lemierre's syndrome. *Case Rep. Infect. Dis.* **2018**, 3628395.

Hashimura, T., Sato, M., Hoshino, E. (2001) Detection of *Slackia exigua*, *Mogibacterium timidum* and *Eubacterium saphenum* from pulpal and periradicular samples using the polymerase chain reaction (PCR) method. *Int. Endod. J.* **34(7)**, 463–470.

Heller, D., Helmerhorst, E., Gower, A., Siqueira, W., Paster, B., Oppenheim, F. (2016) Microbial diversity in the early *in vivo*-formed dental biofilm. *Appl. Environ. Microbiol.* **82**, 1881–1888.

Hill, G. B., Ayers, O. M., Kohan, A. P. (1987) Characteristics and sites of infection of *Eubacterium nodatum*, *Eubacterium timidum*, *Eubacterium brachy*, and other asaccharolytic eubacteria. *J. Clin. Microbiol.* **25(8)**, 1540–1545.

Jaffar, N., Miyazaki, T., Maeda, T. (2016) Biofilm formation of periodontal pathogens on hydroxyapatite surfaces: Implications for periodontium damage. *J. Biomed. Mater. Res. A* **104(11)**, 2873–2880.

Kaplan, C., Ma, X., Paranjpe, A., Jewett, A., Lux, R., Kinder-Haake, S., Shi, W. (2010) *Fusobacterium nucleatum* outer membrane proteins Fap2 and RadD induce cell death in human lymphocytes. *Infect. Immun*. **78**, 4773–4778.

Kawasuji, H., Kaya, H., Kawamura, T., Ueno, A., Miyajima, Y., Tsuda, T., Taniguchi, H., Nakamura, M., Wada, A., Sakamaki, I., Niimi, H., Yamamoto, Y. (2020) Bacteremia caused by *Slackia exigua*: A report of two cases and literature review. *J. Infect. Chemother.* **26(1)**, 119–123.

Kiryu, T., Hoshino, E., Iwaku, M. (1994) Bacteria invading periapical cementum. *J. Endod.* **20(4)**, 169–172.

Kryvtsova, M., Kostenko, Y. (2020) Dominant microbial associations of oral cavity at periodontitis and features of their sensitivity to antibacterial drugs. *Studia Biologica* **14(1)**, 51–62.

Lee, M. Y., Kim, Y. J., Gu, H. J., Lee, H. J. (2016) A case of bacteremia caused by *Dialister pneumosintes* and *Slackia exigua* in a patient with periapical abscess. *Anaerobe* **38**, 36–38.

Leonteva, A., Pototskaya, L., Chervinets, Y. (2023) Mechanisms of oral microbial biofilm formation in healthy people and patients with chronic generalized periodontitis. *Parodontologiya* **3**, *208–217*.

Mark Welch, J. L., Rossetti, B. J., Rieken, C. W., Dewhirst, F. E., Borisy, G. G. (2016) Biogeography of a human oral microbiome at the micron scale. *Proc. Natl. Acad. Sci. U. S. A.* **113(5)**, E791–E800.

Marsh, P. (2004) Dental plaque as a microbial biofilm. *Caries Res.* **38(3)**, 204–211.

Marsh, P. (2005) Dental plaque: Biological significance of a biofilm and community life-style. *J. Clin. Periodontol*. **32(6)**, 7–15.

Marsh, P., Devine, D. (2011) How is the development of dental biofilms influenced by the host? *J. Clin. Periodontol*. **38(11)**, 28–35.

Meyle, J., Chapple, I. (2015) Molecular aspects of the pathogenesis of periodontitis. *Periodontol. 2000* **69(1)**, 7–17.

Moore, W. E. C., Holdeman, L. V., Cato, E. P., Smibert, R. M., Burmeister, J. A., Ranney, R. R. (1983) Bacteriology of moderate (chronic) periodontitis in mature adult humans. *Infect. Immun.* **42(2)**, 510–515.

Okahashi, N., Nakata, M., Terao, Y., Isoda, R., Sakurai, A., Sumitomo, T., Yamaguchi, M., Kimura, R., Oiki, E., Kawabata, S., Ooshima, T. (2011) Pili of oral *Streptococcus sanguinis* bind to salivary amylase and promote the biofilm formation. *Microb. Pathog*. **50(3–4)**, 148–154.

Papapanou, P., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D., Flemmig, T., Garcia, R., Giannobile, W., Graziani, F., Greenwell, H., Herrera, D., Kao, R., Kebschull, M., Kinane, D., Kirkwood, K., Kocher, T., Kornman, K., Kumar, P., Loos, B., Machtei, E., Meng, H., Mombelli, A., Needleman, I., Offenbacher, S., Seymour, G., Teles, R., Tonetti, M. (2018) Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions. *J. Clin. Periodontol*. **89(1)**, 173–182.

Pratt, L. A., Kolter, R. (1998) Genetic analysis of *Escherichia coli* biofilm formation: Roles of flagella, motility, chemotaxis and type I pili. *Mol. Microbiol.* **30(2)**, 285–293.

Rieber, H., Frontzek, A., Schmitt, H. (2019) *Slackia exigua*, an anaerobic Gram-positive rod and part of human oral microbiota associated with periprosthetic joint infection of the hip: First case and review of the literature. *Anaerobe* **56**, 130–132.

Sanz, M., Beighton, D., Curtis, M., Cury, J., Dige, I., Dommisch, H., Ellwood, R., Giacaman, R., Herrera, D., Herzberg, M., Könönen, E., Marsh, P., Meyle, J., Mira, A., Molina, A., Mombelli, A., Quirynen, M., Reynolds, E., Shapira, L., Zaura, E. (2017) Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases: Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. *J. Clin. Periodontol.* **44**, S5–S11 (Suppl. 18).

Sato, T., Hoshino, E., Uematsu, H., Noda, T. (1993) Predominant obligate anaerobes in necrotic pulps of human deciduous teeth. *Microb. Ecol. Health Dis.* **6(6)**, 269–275.

Shahzad, M., Millhouse, E., Culshaw, S., Edwards, C., Ramage, G., Combet, E. (2015) Selected dietary polyphenols inhibit periodontal pathogen growth and biofilm formation. *Food Funct.* **6(3)**, 719–729.

Shemesh, M., Tam, A., Steinberg, D. (2007) Differential gene expression profiling of *Streptococcus mutans* cultured under biofilm and planktonic conditions. *Microbiology (Reading)* **153(Pt 5)**, 1307–1317.

Shemesh, M., Tam, A., Aharoni, R., Steinberg, D. (2010) Genetic adaptation of *Streptococcus mutans* during biofilm formation on different types of surfaces. *BMC Microbiol*. **10**, 51.

Sol, A., Ginesin, O., Chaushu, S., Karra, L., Coppenhagen-Glazer, S., Ginsburg, I., Bachrach, G. (2013) LL-37 opsonizes and inhibits biofilm formation of *Aggregatibacter actinomycetemcomitans* at subbactericidal concentrations. *Infect. Immun.* **81(10)**, 3577–3585.

Stašková, A., Sondorová, M., Nemcová, R., Kačírová, J., Maďar, M. (2021) Antimicrobial and antibiofilm activity of the probiotic strain *Streptococcus salivarius* K12 against oral potential pathogens. *Antibiotics (Basel)* **10(7)**, *793*.

Stepanović, S., Vuković, D., Holá, V., Bonaventura, G., Djukić, S., Cirkovic, I., Růžička, F. (2007) Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS* **115(8)**, 891–899.

Tzu, L., Hall-Stoodley, L., Costerton, J., Stoodley, P. (2004) Bacterial biofilms: From the natural environment to infectious diseases. *Nat. Rev. Microbiol*. **2(2)**, 95–108.

Wade, W. G., Lewis, M. A., Cheeseman, S. L., Absi, E. G., Bishop, P. A. (1994) An unclassified *Eubacterium* taxon in acute dento-alveolar abscess. *J. Med. Microbiol.* **40(2)**, 115–117.