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Effect of D-Ribose on Fusobacterium Nucleatum Planktonic Proliferation and Biofilm Maturation

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ABSTRACT

Purpose: Periodontopathogenic biofilm structure is one of the most important factors in the etiology of inflammatory periodontal diseases. Quorum Sensing inhibitors (QSi) can inhibit biofilm development/maturation by inhibiting bacterial communication mechanism. In this study, we examined the effects of D-ribose (QSi) on the planktonic growth and bio. film formation characteristics of *Fusobacterium nucleatum*, an important periodontopathogenic species that has a binding function on early and late colonization types for the development of periodontopathogenic biofilm.

Methods: The strain of *F. nucleatum* (ATCC 25586) and two clinical isolated strains (AHN 9910 and AHN 9508) were used in all tests. Planktonic proliferation (measured as colony forming units) and established biofilm tests (measured as total biofilm mass) were performed in the presence of 50mM D-ribose.

Results: In planktonic growth tests, statistically significant increase was observed for *F. nucleatum* ATCC 25586 and AHN 9508 strains ($p<0.05$). In established biofilm tests, a mean-based decrease was observed for all species, but a statistically significant difference was found only for *F. nucleatum* AHN 9910.

Conclusions: The fact that the presence of D-ribose can increase the planktonic growth of *F. nucleatum* and its inhibitory effect on biofilm development shows that it may have an adverse effect on biofilm development by disrupting the Quorum-Sensing system.

Keywords: Quorum Sensing, Fusobacterium Nucleatum, Periodontitis, Biofilm

D-Riboz'un Fusobacterium Nucleatum'un Planktonik Çoğalması ve Biyofilm Olgunlaşması Üzerine Etkisi

ÖZET

Amaç: İltihabi periodontal hastalıkların etiyolojisinde periodontopatojenik biyofilm yapısı ana faktörler arasında yer almaktadır. Quorum Sensing inhibitörleri (QSi) bakteriyel haberleşme mekanizmasını inhibe ederek biyofilm gelişimini/olgunlaşmasını engelleyebilmektedir. Biz çalışmamızda D-riboz (QSi) varlığının periodontopatojenik biyofilmin gelişiminde erken ve geç dönem kolonizasyon türlerini bağlayıcı etkiye sahip önemli bir periodontopatojen tür olan Fusobacterium nucleatum'un planktonik çoğalma ve biyofilm oluşturma özellikleri üzerindeki etkilerini araştırdık.

Yöntemler: Tüm testlerde F. nucleatum'un tip suşu (ATCC 25586) ve iki klinik izole suşu (AHN 9910 ve AHN 9508) kullanıldı. Planktonik çoğalma (koloni oluşturan birim olarak ölçüldü) ve yerleşmiş biyofilm testleri (toplam biyofilm kitlesi olarak ölçüldü) 50mM D-riboz varlığında gerçekleştirildi.

Bulgular: Planktonik çoğalma testlerinde 50mM D-Riboz varlığında tüm suşlar için ortalama bazlı artış gözlenmesine rağmen istatistiksel anlamlı artış F. nucleatum ATCC 25586 ve AHN 9508 suşlarında bulundu ($p<0.05$). Yerleşmiş biyofilm testlerinde tüm türler için ortalama bazlı azalma gözlenmekle birlikte istatistiksel anlamlı farklılık yalnızca F. nucleatum AHN 9910 suşunda bulundu.

Sonuç: D-riboz varlığının F. nucleatum' un planktonik çoğalmasını artırabilmesi ve biyofilm gelişiminde neden olduğu inhibe edici etki Quorum-Sensing sistemini bozarak biyofilm gelişiminde olumsuz etki yaratabileceğini göstermektedir.

Anahtar Kelimeler: Quorum Sensing, Fusobacterium Nucleatum, Periodontitis, Biyofilm

Periodontitis is a bacterial disease characterized by inflammation affecting the tissues around the tooth. Considering the periodontal diseases in terms of etiology, the biofilm structure with predominantly anaerobic gram-negative rods is the main factor in this regard (1,2). *F. nucleatum* is a gram-negative species that increases in deep periodontal pockets with a negative health status, which helps the periodontopathogenic biofilm to establish a link between late and early colonization types and accelerates the transition of the biofilm to pathogenicity. *F. nucleatum* can also invade epithelial tissue and cause increased secretion of matrix metalloproteinases (MMPs) in the host tissue; this allows it to play a role in tissue destruction (2,3,5).

Biofilm can be described as self-organizing microbial communities that adhere to living and non-living surfaces and produce extracellular polymeric matrix (4). This organized structure allows bacteria to survive in an environment under stress, to increase their colonization characteristics, and to increase the number of pathogenic bacteria (5,6).

Quorum Sensing (QS) system, which has a major role in biofilm activity, contributes to the establishment of the biofilm order and maintenance of its continuity. Bacteria in the biofilm maintain their communication with each other with the help of the QS system. The LuxS/AI-2 (QS) system can be observed extensively for both gram-negative and gram-positive bacteria. The signal molecule Autoinducer-2 (AI-2) is acknowledged as a general communication language for intra-species and inter-species communication (7,8).

QS inhibitors (QSi) have the potential to significantly reduce bacterial virulence by preventing biofilm formation. Therefore, it can be an important factor for controlling bacterial infection; moreover, they can be an alternative for antibiotics due to this effect (9,10). Ribose components, which are also included in the QS inhibitors, may have an effect on the QS system without toxic effects (11). Since ribose is structurally similar to AI-2, it is thought that it creates an inhibitory effect by creating competition with AI-2 within the biofilm QS system. (12, 13, 14, 15). However, data about the effects of QS inhibitors on periodontopathogenic bacteria and the mechanism of inhibition is still quite limited in the literature. Therefore, the purpose of this study is to examine the planktonic growth and biofilm formation ability of *F. nucleatum*, a periodontopathogenic species commonly found in biofilm under both healthy conditions and periodontal disease conditions, in the presence of QSi (D-ribose).

MATERIAL AND METHOD

Bacterial Species and Cultures

The *F. nucleatum* type strain (ssp. *nucleatum* ATCC 25586) and two clinical isolated strains (ssp. *nucleatum* AHN 9910 and ssp. *nucleatum* AHN 9508) were used in all experiments. Strains were obtained from the Finnish National Institute for Health and Welfare (THL). According to the data from THL database, clinical strain AHN 9508 was isolated from the gingival crevice and clinical strain AHN 9910 was isolated from saliva. Before all experiments, strains were grown on Brucella agars supplemented with Hemin (5mg/l) and vitamin K1 (10mg/l) in anaerobic atmosphere (10% H2, 5% CO_2, 85% N_2 at 37°C, Whitley A35 Anaerobic Workstation, Don Whitley Scientific®, West Yorkshire, UK) for 5 days. Afterwards, colonies collected from agars with the help of sterile cotton swabs were transferred to Bactotrypton liquid medium supplemented with Saccharose (1%), KH_2PO_4 (25 mM) and $MgSO_4$ (4 mM). In order to ensure planktonic growth in the liquid medium, these colonies were kept in anaerobic conditions for 2 days, and the optical densities of all strains were adjusted to 0.5 (OD) at 490 nm, and they were prepared for the tests. It was assumed that this value was equivalent to 4×10^7 CFU/ml (16, 17).

Preparation of QSi Concentration

After commercial purchase of D-ribose (D-(-)-Ribose, Tokyo Chemical Industry®, Tokyo, Japan), it was stored at +4°C until the day of use. D-ribose was prepared as a 200mM stock concentration by dissolving it in liquid medium on the test day in all experiments. In all D-ribose tests, the study groups were diluted with liquid medium to a final concentration of 50mM and the test phase was started afterwards (11, 18).

Planktonic Growth Test

All bacterial strains were set to 0.5 OD (490nm). Control and test groups were prepared in triplicate with a total volume of 200µl. After a 24-hour incubation period under anaerobic conditions, the liquids in eppendorf tubes were diluted for CFU analysis and spread on agar with the help of cotton pellets. Colony numbers were recorded by direct counting after 3-5 days of incubation of the agars under anaerobic conditions. Each test was repeated with the same procedure on three different days.

Covering of 96-Well Plates with Saliva for Stimulation of Biofilm Formation

Saliva was prepared as stock before all experiments. The saliva collected by the primary researcher with the support of paraffin use (Healthy individual, no antibiotic or any drug use in the last 3 months, no smoking) was transferred to a propylene tube and centrifuged at 4°C for 40 minutes at 12,000 g value. Supernatant was collected and transferred to sterile tubes. After the pasteurization process (60° x 30min), it was centrifuged again at 4°C for 40 minutes at 12,000 g value and stored at 4°C until the day of use. The prepared saliva was spread on Brucella agars for sterilization control and incubated for 5 days under aerobic and anaerobic conditions. As a result of sterilization test, no colony formation was observed on Brucella agars.

RESULTS

Planktonic Live Bacteria Growth Results

A statistically significant increase ($p<0.05$) was found for type strain *F. nucleatum* ATCC 25586 and clinical strain *F. nucleatum* AHN 9508 in the presence of 50mM D-ribose in 24-hour planktonic growth tests. Although mean-based increase was observed for *F. nucleatum* AHN9910, no statistically significant difference ($p>0.05$) was found for this strain (Figure 1).

Biofilm Formation (Established) Results

In all established biofilm tests, a decrease in mean-based biofilm mass was observed for all strains of *F. nucleatum*. However, statistically significant decrease in biofilm was found only for clinical strain *F. nucleatum* AHN 9910 among the strains ($p<0.05$) (Figure 2).

DISCUSSION

Pathogenic biofilm structure plays the leading role in the pathogenesis of inflammatory periodontal diseases. *F. nucleatum*, which is in a complex biofilm structure, is a gram-negative anaerobic bacteria species that acts as a bridge between early colonization species and late colonization species and has important effects on the pathogenesis of the disease. Autoinducer-2 (AI-2) signals, one of the communication signals of bacteria, are used by both gram-negative and gram-positive species within the Quorum Sensing system. AI-2 signaling system is also used by *F. nucleatum* (18).

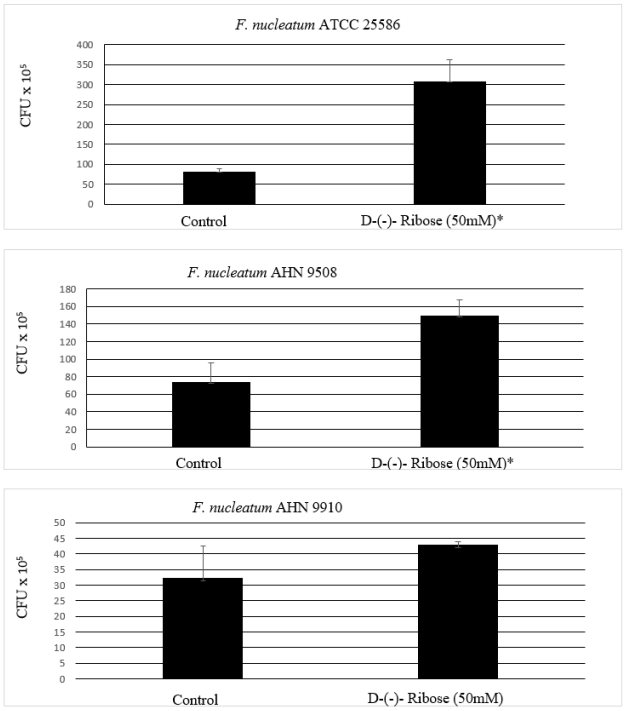

Figure:1 Planktonic growth of three F. nucleatum strains in the D-(-)-Ribose concentration of 50mM. Asterix shows a significant difference compared to control ($p<0.05$)

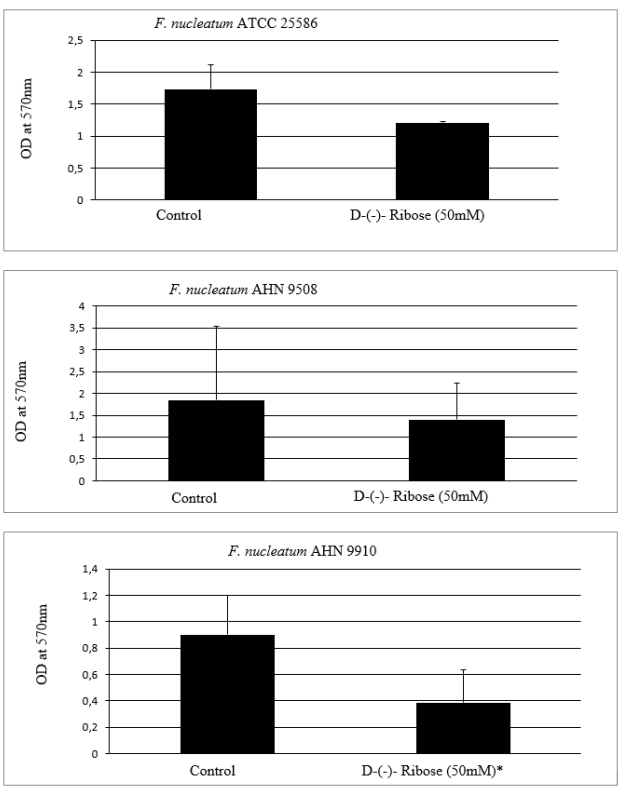

Figure:2 Biofilm formation (established) of three F. nucleatum strains in the D-(-)-Ribose concentration of 50mM. Asteriks shows a significant difference compared to control ($p<0.05$)

DISCUSSION

Pathogenic biofilm structure plays the leading role in the pathogenesis of inflammatory periodontal diseases. *F. nucleatum*, which is in a complex biofilm structure, is a gram-negative anaerobic bacteria species that acts as a bridge between early colonization species and late colonization species and has important effects on the pathogenesis of the disease. Autoinducer-2 (AI-2) signals, one of the communication signals of bacteria, are used by both gram-negative and gram-positive species within the Quorum Sensing system. AI-2 signaling system is also used by *F. nucleatum* (18).

D-ribose, which is one of the Quorum sensing inhibitors, can create inhibition within the QS system by competing with AI-2 signals. One of the most important advantages of D-ribose is that it has minimal toxic side effects (11, 21, 22). Among quorum sensing inhibitors, another frequently used compound in in vitro biofilm studies is Furanone. However, it has been shown that Furanone can have potential toxicity on fibroblasts and it was considered that Furanone compounds may have harmful effects in treatments for humans (19, 20). Therefore, we decided to use D-ribose in the present study.

In this study, the effect of different strains of *F. nucleatum*, which has an important role in the pathogenesis of inflammatory periodontal diseases in the presence of D-ribose, on the mass-based experimental biofilm structure was observed for the first time.

Type strain *F. nucleatum* ATCC 25586 and clinical strains (*F. nucleatum* AHN 9508 and AHN 9910) isolated and stocked from clinical cases were used in order to observe behavioral variances among different strains of the same species in experimental biofilm mass analyzes and planktonic growth tests of *F. nucleatum* (17). We determined the D-ribose concentration we used in our experiments as 50mM, which has been proved to be effective in QS inhibition based on the current literature (13, 18).

Analysis of living bacteria count with CFU, which has also been used in other studies, is acknowledged as the gold standard in the literature (3, 17, 23). We observed a statistically significant increase in *F. nucleatum* ATCC 25586 and *F. nucleatum* AHN 9508 strains in the presence of D-ribose as a result of the planktonic growth tests performed with CFU. For *F. nucleatum* AHN 9910 strain, on the other hand, we did not observe a statistically significant difference, although there was a mean-based increase. D-ribose, which is also included in the digestive system, may play a role as a carbon source for the bacterial microbiota (24, 25). Although ribose uptake and metabolic phosphorylation have been detected in many studies in the literature for many bacterial species, the presence of ribose in the environment may also contribute to the increase in bacterial growth (24, 26). We think that D-ribose, a carbon-derived sugar that is abundant in nature, triggers bacterial growth due to an increase in its metabolic use.

Its biofilm forming ability is very important among the virulence factors of bacteria. Therefore, factors that can inhibit periodontopathogenic biofilm formation are important in terms of the occurrence of periodontal diseases and prevention of the progression of diseases (27, 28). In this study, we decided to apply biofilm formation tests, which were successfully applied in other studies in the literature, for single biofilm tests of *F. nucleatum* (17, 28).

Although mean-based decrease was observed for all *F. nucleatum* strains in established biofilm tests, statistically significant difference was found only for *F. nucleatum* AHN 9910 strain. In the studies in the literature, there are results showing that there may be biofilm inhibition in the presence of D-ribose (11, 18). In the study of Jang et al., it was shown that multiple biofilms formed with *Tannerella forsythia, Treponema denticola* and *Porphyromonas gingivalis,* which are among the important periodontopathogenic bacteria, can be inhibited by D-ribose (18). In the study of Liu L. et al., it was shown that D-ribose can exert dose-dependent antibiofilm activity on *Lactobacillus paraplantarum*, a gram-positive microaerophilic bacterium (11). In the study of Jang et al., it was observed that D-ribose can inhibit dual-biofilm formation using *Streptococcus gordonii*, which is a life-threatening oral bacterial species that can cause infective endocarditis and can coaggregate strongly with *F. nucleatum* (22).

Studies examining the clinical effectiveness of D-ribose have also shown that it can reduce periodontal damage. For example, Ben Amara et al. (29) found in their in-vivo study that the presence of 50mM D-ribose may reduce periodontal damage. In the same study it was also found that the total bacterial count was significantly reduced in the group treated with D-ribose. In the study of Cho et al. (13), they found that the application of D-ribose may reduce periodontal bone destruction.

We think that the presence of D-ribose may have an inhibitory effect on the biofilm formation capacity, which

is an important virulence factor for the occurrence and progression of periodontal diseases. The fact that in the presence of D-ribose can cause an increase in the planktonic growth of *F. nucleatum* and can decrease its biofilm formation capacity, it strengthens the idea that it can disrupt the Quorum Sensing system.

Treatment strategies of periodontopathogenic biofilm-mediated diseases focus on eradication of biofilm by scaling and root planing procedures and sometimes followed by adjunctive use of antiseptics and/or antibiotics. However, chlorhexidine is characterized by discoloration of teeth, bitter taste, and more rarely, mucosal irritation. On the other hand, antibiotics can have various systemic side effects, as well as antibiotic resistance can develop after prolonged use (1, 30). Considering the results of this study and literature data together, D-ribose could be considered as promising alternatives for the adjunctive treatment of biofilm-associated infections. Clinical studies including toxicity analyses as well as multiple biofilm studies with multi-species are required in order to fully explain the effects of Quorum Sensing inhibitors on periodontopathogenic biofilm.

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REFERENCES

1. Socransky SS, Haffajee AD, Dzink JL, et al. Associations between microbial species in subgingival plaque samples. Oral Microbiol Immunol. 1988 Mar;3(1):1-7. doi: 10.1111/j.1399-302x.1988.tb00596.x. PMID: 3268742.
2. Bolstad AI, Jensen HB and Bakken V. Taxonomy, biology, and periodontal aspects of Fusobacterium nucleatum. Clin Microbiol Rev. 1996 Jan;9(1):55-71. doi: 10.1128/CMR.9.1.55. PMID: 8665477; PMCID: PMC172882.
3. Gursoy UK, Pöllänen M, Könönen E, et al. Biofilm formation enhances the oxygen tolerance and invasiveness of Fusobacterium nucleatum in an oral mucosa culture model. J Periodontol. 2010 Jul;81(7):1084-91. doi: 10.1902/jop.2010.090664. PMID: 20350156.
4. Penesyan A, Gillings M and Paulsen IT. Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. Molecules. 2015 Mar 24;20(4):5286-98. doi: 10.3390/molecules20045286. PMID: 25812150; PMCID: PMC6272253.
5. Taylor PK, Yeung AT and Hancock RE. Antibiotic resistance in Pseudomonas aeruginosa biofilms: towards the development of novel anti-biofilm therapies. J Biotechnol. 2014 Dec 10;191:121-30. doi: 10.1016/j.jbiotec.2014.09.003. Epub 2014 Sep 18. PMID: 25240440.
6. Olsen I. Biofilm-specific antibiotic tolerance and resistance. Eur J Clin Microbiol Infect Dis. 2015 May;34(5):877-86. doi: 10.1007/s10096-015-2323-z. Epub 2015 Jan 29. PMID: 25630538.
7. Schauder S, Shokat K, Surette MG, et al. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Mol Microbiol. 2001 Jul;41(2):463-76. doi: 10.1046/j.1365-2958.2001.02532.x. PMID: 11489131.
8. Gospodarek E, Bogiel T and Zalas-Wiecek P. Communication between microorganisms as a basis for production of virulence factors. Pol J Microbiol. 2009;58(3):191-8. PMID: 19899610.
9. Pan J, Ren D. Quorum sensing inhibitors: a patent overview. Expert Opin Ther Pat. 2009 Nov;19(11):1581-601. doi: 10.1517/13543770903222293. PMID: 19732032.
10. Harjai K, Gupta RK and Sehgal H. Attenuation of quorum sensing controlled virulence of Pseudomonas aeruginosa by cranberry. Indian J Med Res. 2014 Mar;139(3):446-53. PMID: 24820840; PMCID: PMC4069740.
11. Liu L, Wu R, Zhang J, et al. D-Ribose Interferes with Quorum Sensing to Inhibit Biofilm Formation of Lactobacillus paraplantarum L-ZS9. Front Microbiol. 2017 Sep 26;8:1860. doi: 10.3389/fmicb.2017.01860. PMID: 29018429; PMCID: PMC5622935.
12. Armbruster CE, Pang B, Murrah K, et al. RbsB (NTHI_0632) mediates quorum signal uptake in nontypeable Haemophilus influenzae strain 86-028NP. Mol Microbiol. 2011 Nov;82(4):836-50. doi: 10.1111/j.1365-2958.2011.07831.x. Epub 2011 Oct 20. PMID: 21923771; PMCID: PMC3609414.
13. Cho YJ, Song HY, Ben Amara H, et al. In Vivo Inhibition of Porphyromonas gingivalis Growth and Prevention of Periodontitis With Quorum-Sensing Inhibitors. J Periodontol. 2016 Sep;87(9):1075-82. doi: 10.1902/jop.2016.160070. Epub 2016 May 13. PMID: 27177290.
14. Brackman G, Celen S, Baruah K, et al. AI-2 quorum-sensing inhibitors affect the starvation response and reduce virulence in several Vibrio species, most likely by interfering with LuxPQ. Microbiology (Reading). 2009 Dec;155(Pt 12):4114-4122. doi: 10.1099/mic.0.032474-0. Epub 2009 Sep 24. PMID: 19778962.
15. Roy V, Meyer MT, Smith JA, et al. AI-2 analogs and antibiotics: a synergistic approach to reduce bacterial biofilms. Appl Microbiol Biotechnol. 2013 Mar;97(6):2627-38. doi: 10.1007/s00253-012-4404-6. Epub 2012 Oct 4. PMID: 23053069.
16. Gursoy UK, Könönen E, Luukkonen N, et al. Human neutrophil defensins and their effect on epithelial cells. J Periodontol. 2013 Jan;84(1):126-33. doi: 10.1902/jop.2012.120017. Epub 2012 Mar 23. PMID: 22443519.
17. Keskin M, Könönen E, Söderling E, et al. Increased proliferation and decreased membrane permeability as defense mechanisms of Fusobacterium nucleatum against human neutrophilic peptide-1. Anaerobe. 2014 Dec;30:35-40. doi: 10.1016/j.anaerobe.2014.08.001. Epub 2014 Aug 12. PMID: 25132418.
18. Jang YJ, Choi YJ, Lee SH, et al. Autoinducer 2 of Fusobacterium nucleatum as a target molecule to inhibit biofilm formation of periodontopathogens. Arch Oral Biol. 2013 Jan;58(1):17-27. doi: 10.1016/j.archoralbio.2012.04.016. Epub 2012 May 26. PMID: 22633049.
19. Trizna, E., Latypova, L., Kurbangalieva, A. et al. 2(5H)-Furanone Derivatives as Inhibitors of Staphylococcal Biofilms. BioNanoSci. 6, 423–426 (2016). https://doi.org/10.1007/s12668-016-0258-1
20. Sharafutdinov IS, Trizna EY, Baidamshina DR, et al. Antimicrobial Effects of Sulfonyl Derivative of 2(5H)-Furanone against Planktonic and Biofilm Associated Methicillin-Resistant and -Susceptible Staphylococcus aureus. Front Microbiol. 2017 Nov 20;8:2246. doi: 10.3389/fmicb.2017.02246. PMID: 29209288; PMCID: PMC5701942.
21. Griffiths JC, Borzelleca JF, St Cyr J. Sub-chronic (13-week) oral toxicity study with D-ribose in Wistar rats. Food Chem Toxicol. 2007 Jan;45(1):144-52. doi: 10.1016/j.fct.2006.08.012. Epub 2006 Aug 30. PMID: 17011102.

22. Kim AR, Ahn KB, Kim HY, Seo HS, Kum KY, Yun CH, Han SH. Streptococcus gordonii lipoproteins induce IL-8 in human periodontal ligament cells. Mol Immunol. 2017 Nov;91:218-224. doi: 10.1016/j.molimm.2017.09.009. Epub 2017 Sep 30. PMID: 28963931.

23. Wilson C, Lukowicz R, Merchant S, et al. Quantitative and Qualitative Assessment Methods for Biofilm Growth: A Mini-review. Res Rev J Eng Technol. 2017 Dec;6(4):http://www.rroij.com/open-access/quantitative-and-qualitative-assessment-methods-for-biofilm-growth-a-minireview-.pdf. Epub 2017 Oct 24. PMID: 30214915; PMCID: PMC6133255.

24. Pokusaeva K, Neves AR, Zomer A, et al. Ribose utilization by the human commensal Bifidobacterium breve UCC2003. Microb Biotechnol. 2010 May;3(3):311-23. doi: 10.1111/j.1751-7915.2009.00152.x. Epub 2009 Oct 15. PMID: 21255330; PMCID: PMC3815373.

25. Walker A, Cerdeño-Tárraga A, Bentley S. Faecal matters. Nat Rev Microbiol. 2006 Aug;4(8):572-3. doi: 10.1038/nrmicro1483. PMID: 16888877.

26. Lopilato JE, Garwin JL, Emr SD, et al. D-ribose metabolism in Escherichia coli K-12: genetics, regulation, and transport. J Bacteriol. 1984 May;158(2):665-73. doi: 10.1128/jb.158.2.665-673.1984. PMID: 6327616; PMCID: PMC215481.

27. Flynn KJ, Baxter NT and Schloss PD. Metabolic and Community Synergy of Oral Bacteria in Colorectal Cancer. mSphere. 2016 May 11;1(3):e00102-16. doi: 10.1128/mSphere.00102-16. PMID: 27303740; PMCID: PMC4888883.

28. Costerton JW, Stewart PS and Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999 May 21;284(5418):1318-22. doi: 10.1126/science.284.5418.1318. PMID: 10334980.

29. Ben Amara H, Song HY, Ryu E, et al. Effects of quorum-sensing inhibition on experimental periodontitis induced by mixed infection in mice. Eur J Oral Sci. 2018 Dec;126(6):449-457. doi: 10.1111/eos.12570. Epub 2018 Sep 19. PMID: 30230039.

30. Gerits E, Verstraeten N, Michiels J. New approaches to combat Porphyromonas gingivalis biofilms. J Oral Microbiol. 2017 Mar 15;9(1):1300366. doi: 10.1080/20002297.2017.1300366. PMID: 28473880; PMCID: PMC5405727.