Antibiofilm effect of probiotic lactic acid bacteria against *Bacillus spp* obtained from the ocular surface

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Abstract. – OBJECTIVE: Increasing emergence of antibiotic resistance has led to developing alternative methods to overcome this issue. The antibiotic resistance is mainly associated with formation of biofilms. Restoring healthy microbiota is one of these methods to fight the biofilm formation. In terms of this, the use of probiotics is a novel approach. In this study, we aimed at investigating the effect of exopolysaccharides (EPSs) of different lactic acid bacteria as probiotics on *Bacillus spp* isolated from the ocular surface, which is known to form biofilms.

MATERIALS AND METHODS: Pathogenic microorganisms were cultivated in "Brain-Hearth infusion" (BHI) broth, and lactic acid bacteria were grown in "De Man, Rogosa, Sharpe" and M17 broth. Molecular identification of lactic acid bacteria was made according to the sequence information of the 16S rRNA gene region. Antimicrobial activity of lactic acid bacteria was determined by sandwich overlay method. The minimum inhibitory concentration values of the exopolysaccharides and antibiofilm activity were determined by microtitration method. For evaluating the effect of EPSs of probiotic bacteria on biofilm, the mean and standard deviations of optical density values were calculated.

RESULTS: The most effective EPS against B. cereus was EPS from *L. rhamnosus 24*, followed by EPS from *L. plantarum* and *L. acidophilus*. The biofilm formation of all pathogenic bacteria that were exposed to probiotic bacteria except *L. rhamnosus 621* and *622* and *L. rhamnosus 3111* was lower than the biofilm formation of the control group.

CONCLUSIONS: EPSs obtained from lactic acid bacteria have antibacterial and antibiofilm activity on pathogenic bacteria isolated from ocular surface. This study is one of the pioneer studies in restoring healthy microbiota on ocular surface with the anticipated forthcoming use of topical probiotics.

Key Words:
Lactic acid bacteria, Ocular surface, Probiotic, Biofilm, Antibiofilm, Antibacterial.

Introduction

Antibiotic resistance is one of the challenging issues of today. Therefore, alternative methods have been sought. Studies have revealed that beneficial bacteria can provide opportunities for the prevention and cure of many diseases. The idea of recreating a healthy microbiota has developed, and accordingly, new treatment and prophylaxis methods have been sought[1]. The use of probiotics is one of these novel modalities. The most important probiotic bacteria are lactic acid bacteria[2,3].

Lactic acid bacteria are beneficial bacteria that have been used in the production of various foods for many years. They are members of the normal microbiota in humans and are often found in association with other microbial species in the digestive and reproductive systems[4,5]. One of the most prominent metabolic products of lactic acid bacteria are EPSs. Exopolysaccharides are usually found on the outside of the microbial cell wall associated with the entire form of polysaccharides. It is known that lactic acid bacterial EPSs have antioxidant, immunomodulatory, anti-inflammatory, anti-biofilm and anti-tumor effects[6-8].

One of the leading causes of antibiotic resistance is the ability of bacteria to form biofilms[9-11]. Biofilm is a structure formed by pathogenic bacteria and bacteria embedded in biofilm are an important virulence factor. Bacteria within the biofilm develop resistance to a variety of conditions and substances, such as antimicrobial agents, temperature, host phagocytes, host oxygen radicals, and proteases[9-13]. This resistance is unique to innate host defenses and biofilm-associated bacteria and differs from conventional antimicrobial resistance. It protects bacteria from antimicrobial agents[14].

Studies on the use of probiotics in eye diseases are limited. In our study, we investigated the

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effectiveness of EPSs of different lactic acid bacteria on *Bacillus spp* and its biofilm isolated from the ocular surface.

Materials and Methods

Test Microorganisms

Our test microorganisms used in the study are *Bacillus spp* obtained from the human ocular surface. These bacteria had been obtained from Eskişehir Technical University, Faculty of Science, stored and used in the studies (Table I). Probiotic bacteria are lactic acid bacteria of human origin (coded 24, 311, 71, 11, 321, 622, 3111, 621) (Table II).

Preparation of Microorganisms

Pathogenic microorganisms were removed from the stock and were cultivated in "Brain-Hearth infusion" (BHI) broth. Pathogenic bacteria were incubated at 37°C for 24 hours. Lactic acid bacteria were grown in "De Man, Rogosa, Sharpe" (MRS) and M17 broth. They were incubated at 37°C for 24-48 hours in an environment containing 10% CO_2. After the cultures developed, pathogenic bacteria were replanted on BHI agar and blood agar and were multiplied by incubating at 37°C for 24 hours. Lactic acid bacteria were grown by inoculating on MRS agar and M17 agar and were incubated for 24-48 hours at 37°C in an environment containing 10% CO_2. The purity of the developing colonies was checked by their morphology and Gram staining.

Identification of Lactic Acid Bacteria

Molecular identification of bacteria was made according to the sequence information of the 16S rRNA gene region. After bacterial DNA isolation PCR was established for the 16S rRNA gene region using 27F:5_AGAGTTTGATCMTGGCTCAG-3'; 1492R:5_TACGGYTACCTTGTTACGACTT-3 primers[15].

Table I. Pathogenic bacteria isolated from ocular surface.

Code of microorganism	Name of Microorganism
13/2	*Bacillus cereus*
20PCA	*Bacillus cereus*
23PCA	*Bacillus pumilus*
35-1 PCA	*Bacillus spp.*
24-1	*Bacillus cereus*
13/2 PCA	*Bacillus cereus*
8/2PCA	*Bacillus agri*

After obtaining the PCR products, they were purified. Sequence analysis was performed by Macrogen Europe company (Amsterdam, The Netherlands). The 16S rRNA gene sequences obtained as a result of the sequence analysis were arranged using the BioEdit program (Version 7.2, Thomas A. Hall, CA, USA). The sequences read with the primers 27F and 1492R were combined and compared with other 16S rRNA sequences in the GenBank database available on the National Center for Biotechnology (NCBI) website using the The Basic Local Alignment Search Tool (BLAST). Identification of isolates at the species level was determined by percentage similarity.

Obtaining Exopolysaccharide from Lactic Acid Bacteria

Lactic acid bacteria were incubated in MRS broth for 24-48 hours at 35°C under 10% CO_2 conditions. After incubation, the cultures were centrifuged at 6000 rpm at +4°C for 20 minutes and the supernatant was transferred to a different tube. 20 % trichloro acetic acid was added to the tube and left overnight at +4°C. After this period, the samples were centrifuged at 10000 rpm at +4°C for 30 minutes. After centrifugation, the liquid formed in the upper part was transferred to another tube. Cooled ethanol was added to the liquid and left at -20°C overnight. Then the samples were centrifuged at 10000 rpm at +4°C for 30 minutes. After centrifugation, the liquid part formed in the upper part was poured out and hot distilled water was added on the pellet formed at the bottom. The pellet was dissolved and used in the studies.

Lactic Acid Bacteria and EPS as Antimicrobials

Antimicrobial activity of lactic acid bacteria was determined by sandwich overlay method. The minimum inhibitory concentration (MIC) values of the prepared exopolysaccharides were determined by microtitration method.

Determination of Antimicrobial Activity by Sandwich Overlay Method

For this purpose, 20 ml of MRS agar was distributed on petri dishes. After the agar solidified, 10 µl from cultures of lactic acid bacteria incubated for 18-24 hours were cultivated. Afterwards, it was incubated for 24-48 hours at 37°C in an environment containing 10% CO_2. Then, 7 ml of BHI soft agar prepared with 106 cfu per milliliter of pathogenic bacteria was poured on it and

Table II. Species and GenBank accession numbers of lactic acid bacterial isolates.

Code of bacteria	Name of bacteria	Access No
24	*L. rhamnosus*	KM513646.1
311	*L. brevis*	CP021674
71	*L. plantarum*	MK027021
11	*L. acidophilus*	CP010432
321	*L. rhamnosus*	KM513646.1
622	*L. rhamnosus*	LT220504.1
3111	*L. rhamnosus*	KM513646.1
621	*L. rhamnosus*	KP090128.1

spread over the growing cultures in the petri dish. Petri dishes were incubated at 37°C for 24 hours. Then, zone diameters formed after incubation were evaluated. Studies were carried out in double parallel[16].

Determination of Antibiofilm Activity of Exopolysaccharides

Antibiofilm activity of exopolysaccharides was determined by microtitration method. EPS solutions of lactic acid bacteria were sterilized in an autoclave at 110°C for 10 minutes. Pathogenic test bacteria were inoculated in BHI broth medium and incubated at 37°C for 24 hours. They were transferred to tryptic soy broth (TSB) containing 2 % glucose. Its density was adjusted according to McFarland 1, and 100 µl was inoculated to the microtitration plate. After planting, 100 µl of the solution containing 20 mg/ml EPS was transferred. The biofilm was determined after 24 hours of incubation at 37°C.

Determination of Biofilm

After the completion of incubation period of the pathogenic bacteria, the plates were evaluated by reading at 490 nm in an enzyme-linked immunosorbent assay (ELISA) reader. Then, the microtitration plates were emptied and washed thrice with sterile physiological saline (FTS) water. The planktonic bacteria were removed and the bacteria that formed a biofilm on the plate wall remained. After the plates dried, the wells were treated with 200 µl of 96% methanol for 5 minutes. Thus, the fixation of biofilm-forming bacteria was achieved. The methanol was then drained, and the plates were left to dry. 200 µl of 1% crystal violet was added to the wells and incubated for 5 minutes at room temperature. After washing the dye gently, 200 µl of 33% acetic acid was poured on and optical density was measured at 570 nm. As negative control, medium free of bacteria was used[17,18]. The study was performed in pairs in parallel.

Preparation and Image Acquisition of Samples for Scanning Electron Microscopy (SEM)

After washing the samples with 0.1 M cacodylate buffer, they were fixed with 2.5% glutaralde-

Table III. MIC (mg/mL) values of exopolysaccharides of lactic acid bacteria on pathogenic bacteria.

Pathogen bacteria	Minimum inhibitory concentration (mg/mL)							
	L. rhamnosus 321	*L. acidophilus*	*L. brevis*	*L.rhamnosus 3.1.1.1*	*L. rhamnosus 24*	*L. plantarum 71*	*L. rhamnosus 621*	*L. rhamnosus 622*
*B. cereus*13/2	-	5,000	**4,500**	6,750	5,750	7,000	8,250	6,750
*B. cereus*20PCA	-	**2,500**	9,000	3,375	5,750	7,000	4,125	3,375
B. pumilus 23PCA	3,625	5,000	9,000	3,375	**1,438**	**1,750**	4,125	3,375
B. agri 8/2PCA	-	5,000	4,500	6,750	5,750	7,000	8,250	6,750
Bacillus spp. 35-1 PCA	7,250	**2,500**	4,500	3,375	**1,438**	**1,750**	4,125	6,750
*B. cereus*24-1	7,250	**1,250**	9,000	1,688	**0,719**	**0,875**	8,250	3,375
B. cereus 13/2 PCA	7,250	-	9,000	1,688	1,438	1,750	8,250	3,375

Table IV. Mean optic density measurements of biofilm with probiotic bacteria.

Name of probiotic bacteria	Mean optic density measurement (Mean±SD)
Control	0.38±0.10
L. rhamnosus 24	0.06±0.02
L. brevis 311	0.10±0.10
L. plantarum 71	0.03±0.03
L. acidophilus 11	0.04±0.03
L. rhamnosus 321	0.18±0.13
L. rhamnosus 622	0.10±0.10
L.rhamnosus 3111	0.31±0.14
L. rhamnosus 621	0.24±0.16

hyde for 1-1,5 hours at room temperature. After fixation, the samples were washed again with cacodylate buffer. Post-fixation was maintained for 1 hour with 1% OsO_4. It was washed again 2-3 times with Cacodylate buffer. Dehydration was achieved with 30%, 50%, 70%, 90% and 100% alcohol series. This process was repeated twice. Drying was carried out in the Critical Point Dryer immediately after the alcohol series. Afterwards, the samples were coated with gold at 40 mA for 1 minute and examined in SEM.

Statistical Analysis

The mean and standard deviations of optical density values were calculated using descriptive statistical methods. Percentage changes were calculated. The data were evaluated using the SPSS program (IBM Corp. 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY, USA).

Results

Identification and accession numbers of lactic acid bacteria of human origin according to the 16S rRNA gene region are shown in Table II. Five of the selected isolates were *Lactobacillus rhamnosus*, one was *Lactobacillus acidophilus*, one was *Lactobacillus brevis*, and one was *Lactobacillus plantarum*. Lactic acid bacteria were shown to exert varying degrees of antibacterial activity to pathogenic test bacteria.

The MIC values of EPSs of different lactic acid bacteria on pathogenic bacteria were calculated (Table III). The values ranged from 9.0 mg/mL to 0.719 mg/mL. The most effective EPS against *B. cereus* was EPS from *L. rhamnosus* 24, followed by EPS from *L. plantarum* and *L. acidophilus.* Exopolysaccharides applied at the MIC values inhibited the biofilm formation of the pathogenic test bacteria at different rates. The optical density measurements of *L. rhamnosus* 3111 were found to be similar to those of control group. The mean optical density measurements of other probiotic bacteria were lower than the control group. The lowest mean optical density was obtained by *L.plantarum, L.acidophilus, L.rhamnosus 24* (Table IV). Percentage changes of optical density measurements were compared to the control group (Table V). Biofilm formation was found to

Table V. Mean optic density measurements of biofilm with probiotic bacteria.

PCA	*Bacillus spp.* 35-1	*B.cereus* 13/2	*B.cereus* 20PCA	*B.cereus* 24-1	*B.cereus* 13/2 PCA	*B.pumilus* 23PCA	*B.agri* 8/2PCA
L.rhamnosus 321	59.7	46.2	55.7	97.4	71.7	7.2	37.9
L. rhamnosus 3111	-51.5*	51.2	41.3	11.1	86.9	23.1	-112.3*
L.rhamnosus 622	-69.4*	-11.9*	35.3	52.3	0.1	17.7	9.6
L. rhamnosus 621	-17.5*	69.4	72.2	18.1	-1.6*	71.8	62.9
L.rhamnosus 24	66.3	85.5	98.2	80.1	88.9	87.1	88.1
L. plantarum	91.7	98.4	75.6	89.2	96.5	95.4	87.9
L. brevis	32.9	64.1	86.4	99.1	86.9	95.7	94.1
L. acidophilus	92.4	94.1	72.3	87.5	97.3	94.6	75.8

*Positive values are % lower than control group, negative values are % higher than control group.

be higher in *Bacillus spp* 35-1PCA and B. agri18/2 PCA using *L.rhamnosus 3111* than in the control group. In *L. rhamnosus 621*, biofilm production of *Bacillus spp* 35-1PCA and *B.cereus* 13/2 PCA was found to be higher than the control group. In *L. rhamnosus 622*, biofilm production of *Bacillus spp* 35-1PCA and *B. cereus* 13/2 was found to be higher than the control group. The effect of all other probiotic bacteria on all pathogenic bacteria in terms of biofilm formation was found to be lower than the control group. These data were also confirmed by SEM images (Figure 1).

Discussion

The human body has been colonized with more than 100 trillion microorganisms[19]. The regions where microbial colonization is most intense are the digestive system, oral cavity, skin and vagina[20]. Beneficial microorganisms have an important place in this diversity. A recent study reported that the mix of *Bifidobacterium lactis*, *Lactobacillus salivarius* and *Lactobacillus acidophilus* has an anti-inflammatory effect in acute uncomplicated diverticulitis[21]. In an experimental study, it was shown that administration of *Lactobacillus rhamnosus* could attenuate the formation of atherosclerotic lesions in ApoE$^{-/-}$ mice[22]. Although it is thought that there are not so many microorganisms on the ocular surface, independent studies have shown the microbial diversity of the eye surface[23,24]. Studies have shown that balance-providing microbiomes reduce pathological bacterial colonization on the eye surface[24-26]. Iovieno et al[27] applied *Lactobacilus acidophilus* topically in patients with vernal keratoconjunctivitis for 4 weeks and found that the symptoms improved in 6 of 7 patients. Chisari et al[28] divided dry eye patients into 2 groups. They used artificial tears for one group, artificial tears for the other group, and a mixture of *Bifidobacterium lactis* and *Bifidobacterium bifida* microorganisms. They reported that probiotic bacteria may be helpful in the treatment of dry eye. Feher et al[29] reported that when systemic probiotic lysate is added to orally taken omega 3 fatty acids and Vit A, B, D, it has a greater effect on irritable eye syndrome. They stated that probiotics with nanoparticles are more effective. Dennis-Wall et al[30] applied*, Bifidobacterium bifidum*, *Bifidobacterium longum* and *Lactobacillus gasseri* systemically to patients who had allergic rhinoconjunctivitis. They stated that the quality-of-life questionnaires of rhinoconjunctivitis patients were better. Nehal et al[31] reported that EPS of *Lactococcus lactis* obtained from camel milk showed inhibitory effects against *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Listeria monocytogenes, Bacillus cereus, Proteus mirabilis, Enterobacter cloacae, Acinetobacter baumannii.* De Grandi et al[32] reported that intranasal administration of *Streptoccoccus salivarius* and *Streptoccoccus oralis* temporarily modulated nasal microbiota. However, in those studies the source of pathogenic bacteria was not ocular surface. In our study, we investigated the anti-microbial and anti-biofilm effects of EPSs produced by probiotic bacteria against *Bacillus spp*. The most effective EPSs against *Bacillus* obtained from the eye surface belonged to *L. rhamnosus 24*, followed by EPSs belonging to *L. plantarum* and *L. acidophilus*.

Another virulence factor that determines the pathogenicity of bacteria is the biofilm. With its formation, pathogenic bacteria become resistant to antibiotics. As a physical barrier, biofilm prevents antibiotics and disinfectants from reaching the microorganism cell[33,34]. The bacterial density of biofilm microcolonies alters the microenvironment by waste production. The antimicrobial ac-

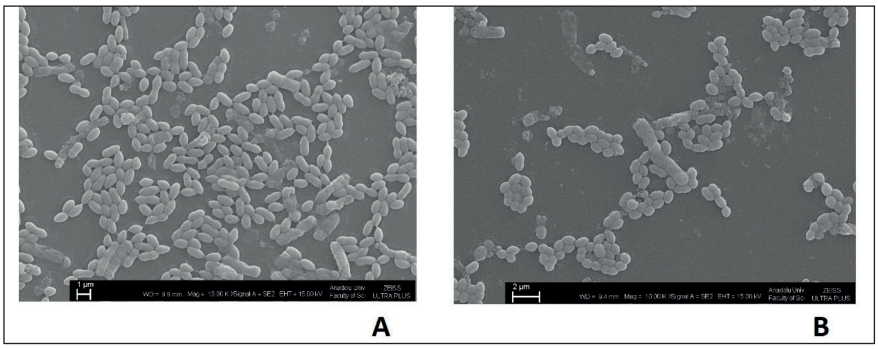

Figure 1. SEM images of antibiofilm activity. A-B Effect of EPS formed by *L.rhamnosus* on B. cereus biofilm formation; No EPS (**A**), with EPS (**B**).

tivity of aminoglycosides is decreased with lack of oxygen[35,36]. In previous studies, some microorganisms isolated from the conjunctiva were shown to have antimicrobial activity against pathogenic bacteria[37]. However, the literature showing the effects of probiotic bacteria against biofilm formation is limited. Mahdhi et al[3] reported that EPS of *L. plantarum* and *Bacillus spp* inhibited biofilm formation of *E. coli*. They attributed this to a decrease in the level of indole production due to signal molecules and a decrease in hydrophobicity. *L. acidophilus* –EPS HT-29 inhibited the adhesion of *E. coli* 0157:H7 to human colon adenocarcinoma cells[2]. While there are studies regarding substances and drugs effective on biofilms of pathogens on ocular surface, this study is the first one evaluating the effect of probiotic bacteria used in this sense. In previous studies, antimicrobials such as vancomycin, linezolid, imipenem, and anti-inflammatory drugs were applied on biofilms formed by pathogenic bacteria obtained from the eye surface, however, the effects of these drugs were limited[38,39]. In this study, EPSs obtained from probiotics were used. The most effective EPSs belonged to *L.rhamnosus* 24, *L.brevis, L.plantarum* and *L.acidophilus*. It was also noted that some EPSs trigger biofilm formation in some bacteria at a very small rate.

Conclusions

EPSs obtained from lactic acid bacteria have antibacterial and antibiofilm activity. In particular, this preliminary study is one of the first studies evaluating the effect of probiotics on a specific bacterium and its biofilm isolated from the eye surface. The findings may aid for the selection of probiotic to be used in forthcoming studies. The possibility of establishing a homeostasis between the topical drops to be developed and the resident microbiota and local immune defenses on the ocular surface is crucial for ocular surface health.

Acknowledgements

Part of this study was presented as a free paper in Turkish Ophthalmological Association Annual Meeting in 2019. We thank to Merve Kaya for her assistance in acquisition of the data.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1) Lu LJ, Liu J. Human microbiota and ophthalmic disease. Yale J Biol Med 2016; 89: 325-330.
2) Kim Y, Oh S, Kim SH. Released exopolysaccharide (r-EPS) produced from probiotic bacteria reduce biofilm formation of enterohemorrhagic Escherichia coli O157:H7. Biochem Biophys Res Commun 2009; 379: 324-329.
3) Mahdhi A, Leban N, Chakroun I, Bayar S, Mahdouani K, Majdoub H, B. Kouidhi B. Use of extracellular polysaccharides, secreted by Lactobacillus plantarum and Bacillus spp., as reducing indole production agents to control biofilm formation and efflux pumps inhibitor in Escherichia coli. Microb Pathog 2018; 125: 448-453.
4) Dinçer E, Kıvanç M, Karaca H. Lactic acid bacteria as biopreservative and bacteriocins. Gıda 2010; 35: 55-62.
5) Rademaker JLW, de Brujin FJ. Characterization and classification of microbes by rep-PCR genomic fingerprinting and computer-assisted patern analysis. Available at: http//www.msu.edu.edu./user/debruıjn/dna1- 4htm, Nov 2000.
6) Van Casteren WHM, Dijkema C, Schols H A, Beldman G, Voragen AGJ. Characterisation and modification of the exopolysaccharide produced by Lactococcus lactis subsp. cremoris B40. Carbohydr Polym 1998; 37: 123-130.
7) Champagne CP, Gardner NJ, Lacroix C. Fermentation technologies for the production of exopolysaccharide synthesizing Lactobacillus rhamnosus concentrated cultures. J Biotechnol 2007; 10: 211-220.
8) Zhoua Y, Cuia Y, Qub X. Exopolysaccharides of lactic acid bacteria: Structure, bioactivity and associations: A review. Carbohydrate Polymers 2019; 207: 317-332.
9) Kıvanç SA, Kıvanç M, Kılıç V, Güllülü G, Özmen AT. Comparison of biofilm formation capacities of two clinical isolates of Staphylococcus Epidermidis with and without icaA and icaD genes on intraocular lenses. Turk J Ophthalmol 2017; 47: 68-73.
10) Kıvanç SA, Kıvanç M, Yiğit T. Antibiotic susceptibility, antibacterial activity and characterisation of Enterococcus faecium strains isolated from breast milk. Exp Ther Med 2016; 12: 1732-1740.
11) Kıvanç SA, Kıvanç M, Bayramlar H. Microbiology of corneal wounds after cataract surgery: biofilm formation and antibiotic resistance patterns. J Wound Care 2016; 25: 14-19.
12) Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbial Rev 2002; 15: 167-193.
13) Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis 2002; 8: 881-890.
14) Anderl JN, Franklin MJ, Stewart PS. Role of antibiotic penetration limitation in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin. Antimicrob Agents Chemother 2000; 44: 1818-1824.
15) Pratten, J, Wilson M, Spratt DA. Characterization of in vitro oral bacterial biofilms by traditional and molecular methods. Oral Microbiol Immunol 2003; 18: 45-49.

16) González L, Sandoval H, Sacristán N, Castro JM, Fresno JM, Tornadijo ME. Identification of lactic acid bacteria isolated from Genestoso cheese throughout ripening and study of their antimicrobial activity. Food Control 2007; 18: 716-722.
17) Rossoni RD, de Barros PP, de Alvarenga JA, Ribeiro FC, Velloso MDS, Fuchs BB, Mylonakis E, Jorge AOC, Junqueira JC. Antifungal activity of clinical Lactobacillus strains against Candida albicans biofilms: identification of potential probiotic candidates to prevent oral candidiasis. Biofouling 2018; 34: 212-225.
18) 18- Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods 2000; 40:175-179.
19) Wang B, Yao M, Lv L, Ling Z, Li L. The Human microbiota in health and disease. Engineering 2017; 3: 71-82.
20) Takahashi N. Microbial ecosystem in the oral cavity: metabolic diversity in an ecological niche and its relationship with oral diseases. Int Congress Series 2005; 1284: 103-112.
21) Petruzziello C, Marannino M, Migneco A, Brigida M, Saviano A, Piccioni A, Franceschi F, Ojetti V. The efficacy of a mix of three probiotic strains in reducing abdominal pain and inflammatory biomarkers in acute uncomplicated diverticulitis. Eur Rev Med Pharmacol Sci 2019; 23: 9126-9133.
22) Fang Y, Chen HQ, Zhang X, Zhang H, Xia J, Ding K, Fang ZY. Probiotic administration of lactobacillus rhamnosus GR-1 attenuates atherosclerotic plaque formation in ApoE-/- mice fed with a high-fat diet. Eur Rev Med Pharmacol Sci 2019; 23: 3533-3541.
23) Dong Q, Brulc JM, Iovieno A, Bates B, Garoutte A, Miller D, Revanna KV, Gao X, Antonopoulos DA, Slepak VZ, Shestopalov VI. Diversity of bacteria at healthy human conjunctiva. Invest Ophthalmol Vis Sci 2011; 52: 5408-5413.
24) Browne HP, Forster SC, Anonye BO, Kumar N, Neville BA, Stares MD, Goulding D, Lawley TD. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. Nature 2016; 533: 543-546.
25) Kugadas A, Wright Q, Geddes-McAlister J, Gadjeva M. Role of microbiota in strengthening ocular mucosal barrier function through secretory IgA. Invest Ophthalmol Vis Sci 2017; 58: 4593-600.
26) Kugadas A, Gadjeva M. Impact of microbiome on ocular health. Ocul Surf 2016; 14: 342-349.
27) Iovieno A, Lambiase A, Sacchetti M, Stampachiacchiere B, Micera A, Bonini S. Preliminary evidence of the efficacy of probiotic eye-drop treatment in patients with vernal keratoconjunctivitis. Graefes Arch Clin Exp Ophthalmol 2008; 246: 435-441.
28) Chisari G, Chisari EM, Francaviglia A, Chisari CG. The mixture ofbifidobacterium associated with fructo-oligosaccharides reduces the damage of the ocular surface. Clin Ter 2017; 168: e181-185.
29) Feher J, Pinter E, Kovács I, Helyes Z, Kemény A, Markovics A, Plateroti R, Librando A, Cruciani F. Irritable eye syndrome: neuroimmune mechanisms and benefits of selected nutrients. Ocul Surf 2014; 12: 134-145.
30) Dennis-Wall JC, Culpepper T, Nieves C Jr, Rowe CC, Burns AM, Rusch CT, Federico A, Ukhanova M, Waugh S, Mai V, Christman MC, Langkamp-Henken B. Probiotics (Lactobacillus gasseri KS-13, Bifidobacterium bifidum G9-1, and Bifidobacterium longum MM-2) improve rhinoconjunctivitis-specific quality of life in individuals with seasonal allergies: a double-blind, placebo-controlled, randomized trial. Am J Clin Nutr 2017; 105: 758-767.
31) Nehal F, Sahnoun M, Smaoui S, Jaouadi B, Bejar S, Mohammed S. Characterization, high production and antimicrobial activity of exopolysaccharides from Lactococcus lactis F-mou. Microbial Pathogenesis 2019; 132: 10-19.
32) De Grandi R, Bottagisio M, Di Girolamo S, Bidossi A, De Vecchi E, Drago L. Modulation of opportunistic species Corynebacterium diphtheriae, Haemophilus parainfluenzae, Moraxella catarrhalis, Prevotella denticola, Prevotella melaninogenica, Rothia dentocariosa, Staphylococcus aureus and Streptococcus pseudopneumoniae by intranasal administration of Streptococcus salivarius 24SMBc and Streptococcus oralis 89a combination in healthy subjects. Eur Rev Med Pharmacol Sci 2019; 23: 60-66.
33) Shih PC, Huang CT. Effect of quorum-sensing deficiency on Pseudomonas aeruginosa biofilm formation and antibiotic resistance. J Antimicrob Chemother 2002; 49: 309-314.
34) Drenkard E, Ausubel FM. Pseudomonas biofilm formation and antibiotic resistance are linked to phenotypic variation. Nature 2002; 416: 740-743.
35) Tack KJ, Sabath LD. Increased minimum inhibitory concentrations with anaerobiasis for tobramycin, gentamicin, and amikacin, compared to latamoxef, piperacillin, chloramphenicol, and clindamycin. Chemotherapy 1985; 31: 204-210.
36) del Pozo JL, Patel R. The challenge of treating biofilm-associated bacterial infections. Clin Pharmacol Ther 2007; 82: 204-209.
37) Kıvanç SA, Takım M, Kıvanç M, Güllülü G. Bacillus Spp. isolated from the conjunctiva and their potential antimicrobial activity against other eye pathogens. Afr Health Sci 2014; 14: 364-371.
38) Kivanc SA, Akova-Budak B, Yildiz M, Kivanc M. The effect of the linezolid and the vancomycine on biofilm production that formed on two different acrylic hydrophobic intraocular lenses. Invest Ophthalmol Vis Sci 2015; 56: 287.
39) Kivanc SA, Akova-Budak B, Kivanc M, Cevik SG, Gullulu G, Ozmen AT, Yucel AA. The effects of sub- and above-MIC concentrations of vancomycin, linezolid and imipenem on Staphylococcus spp. isolated from ocular surface. Invest Ophthalmol Vis Sci 2016; 57: 5401.