Scientific Research Publishing

Advances in Infectious Diseases, 2025, 15(3), 594-604
https://www.scirp.org/journal/aid
ISSN Online: 2164-2656
ISSN Print: 2164-2648

Anti-Biofilm Effect of Cannabinoids on a Clinical Isolate of *Pseudomonas aeruginosa*

Adina Kagan[1], Summer Rotering[1], Zvi G. Loewy[1,2*]

[1]Touro College of Pharmacy, Touro University, New York, USA
[2]School of Medicine, New York Medical College, New York, USA
Email: *zvi.loewy@touro.edu

How to cite this paper: Kagan, A., Rotering, S., and Loewy, Z.G. (2025) Anti-Biofilm Effect of Cannabinoids on a Clinical Isolate of *Pseudomonas aeruginosa*. *Advances in Infectious Diseases*, **15**, 594-604.
https://doi.org/10.4236/aid.2025.153045

Received: August 25, 2025
Accepted: September 21, 2025
Published: September 24, 2025

Abstract

Antimicrobial resistance is a significant global health issue. The prevalence and spread of antimicrobial-resistant organisms have been identified by the World Health Organization as one of the major healthcare challenges. Biofilms, communities of microorganisms largely resistant to antibiotics, compound the antimicrobial-resistant challenge. Exacerbation of chronic obstructive pulmonary disease is manifested by microbial infection. The objective of this study was to identify natural compounds that have antimicrobial activity and the potential to mitigate COPD exacerbation. Two cannabinoids, Cannabidiol (CBD) and Cannabigerol (CBG), were evaluated for antimicrobial activity on *Pseudomonas aeruginosa* strain PA14, one of the most common bacteria implicated in acute exacerbation of COPD. Both CBD and CBG showed no activity on planktonic PA14 cells. CBD and CBG, individually as well as in combination, inhibited and eradicated PA14 biofilms. This study demonstrates that CBD and CBG may be viable approaches to consider for biofilm-based infections.

Keywords

Cannabidiol, Cannabigerol, Pseudomonas Aeruginosa, Biofilm Inhibition, Biofilm Exacerbation

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death globally. COPD includes two clinical phenotypes, emphysema and chronic bronchitis. Initially, factors that contribute to the onset of COPD include genetic factors, pollution, cigarette smoke, and exposure to diverse chemicals. Subsequently, exacerbation of COPD correlates with bacterial colonization and respir-

atory viral infections. The primary bacterial pathogens implicated in COPD exacerbation include *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*.

Pathogenic bacteria can survive in the lungs of COPD patients for several months. This may be attributed to the formation of biofilms, which may also be responsible for recurring episodes of acute exacerbations [1]. Biofilms are dense micro-communities, often attached to inert surfaces, that are encapsulated by secreted polymers [2]. The development of a biofilm is a dynamic process [3]. Initially, a planktonic microbe attaches itself to a surface, and it then subsequently aggregates with other microbes in the formation of a biofilm.

Patients who suffer from exacerbations frequently experience poor quality of life, accelerated decline in lung function, increased healthcare expenses, and increased morbidity and mortality. Studies testing patients' sputum cultures have determined that approximately 50% of COPD exacerbations are attributed to bacterial lung infections [4].

The Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) publishes guidelines that are widely accepted by clinicians in the United States and internationally as the standard on which to base therapy. The guidelines offer recommendations on how to initiate therapy in patients newly diagnosed with COPD, as well as recommendations on how to modify therapy in patients who experience frequent exacerbations. The GOLD guidelines offer recommendations regarding how to treat acute exacerbations, including antimicrobials, mucoregulators, and the use of bronchodilators and anti-inflammatory drugs [5].

Natural products have consistently served as a valuable source for antimicrobial drug candidates [6]. In recent years, studies have been performed to evaluate the medicinal applications of cannabinoids, which are found in *Cannabis* plants. Results of numerous studies highlight the potential antimicrobial applications of multiple substances found in *Cannabis* plants [7]. Cannabinoids can be segmented as either endogenous or exogenous [8]. In the treatment of bacterial infections, results from animal studies demonstrated that exogenous cannabinoids, particularly Tetrahydrocannabinol (THC), can minimize resistance to numerous pathogens, including *Listeria monocytogenes*, *Treponema pallidum*, *Legionella pneumophila*, and *Staphylococcus aureus* [9]. Cannabidiol (CBD), another exogenous cannabinoid, has been studied in numerous *in vitro* studies. Results of these studies demonstrated that CBD has both bacteriostatic and bactericidal activity against several bacteria, including methicillin-susceptible *Staphylococcus aureus* (MSSA), Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Streptococcus mutans*, and *Streptococcus faecalis* [6] [7]. In a study conducted by Luz-Veiga *et al.*, CBD displayed antimicrobial effects against *Pseudomonas aeruginosa* and *Escherichia coli* [10]. Inhibition and eradication of *P. aeruginosa* biofilms with CBD have been demonstrated on soft contact lenses [11]. Promising antibacterial activity with CBD has been reported with *Salmonella typhimurium* and *Salmonella newington* [12]. Anti-biofilm activity of CBD has also been demonstrated

for the fungal pathogen *Candida albicans* [13]. Studies with Cannabigerol (CBG) have shown anti-biofilm activity with *S. mutans*, and quorum sensing and biofilm formation of *Vibrio harveyi* were reduced in the presence of CBG [14] [15]. Cannabinoids can also be useful for enhancing the activity of antibiotics when used together with antibiotics [16]. Overall, these results highlight the broad applications of cannabinoids as antimicrobial therapeutic alternatives.

The most common bacterium implicated in acute exacerbation of COPD (AECOPD) is *Pseudomonas aeruginosa*, which is a gram-negative, aerobic species that is responsible for opportunistic infections in immunocompromised patients [17]. *P. aeruginosa* excels in its ability to form biofilms. Due to the difficulty of delivering antibiotics directly to the bacteria, these biofilms pose a significant challenge for treating infections caused by this organism. *P. aeruginosa's* biofilm-forming abilities reinforce the need for novel pharmacotherapies that exhibit activity against this pathogen. There are multiple strains of *P. aeruginosa* that differ from one another in their genome, growth conditions, and mechanisms of biofilm formation. PA14 is one of the most well-studied strains of *P. aeruginosa* and holds particular importance in research as it is a clinical isolate of this bacterial species. Although PA14 is more virulent, its genome is highly conserved with that of PA01. We therefore chose PA14 for this study to gain insight into the potential clinical utility.

This study focused on investigating the activity of CBD and CBG on PA14 biofilms. Parallel assays were performed with CBD and CBG to assess inhibition as well as eradication of PA14 biofilms. The study also evaluated CBD and CBG combination formulations on both PA14 inhibition and eradication.

2. Materials and Methods

2.1. Bacterial Strains, Growth Media, Conditions, and Materials

The *P. aeruginosa* strain PA14 used in this study was a gift from Dr. Wen Shi, Forsyth Institute/Harvard Dental School. Tryptic Soy Agar (TSA) and Tryptic Soy Broth (TSB) media (Becton Dickinson Company, Sparks, MD, USA) were used to culture the planktonic form of PA14. PA14 was incubated with agitation at 37˚C for 24 hours until absorbance at OD_{490nm} was ≥ 1. CBD and CBG at concentrations of 1 mg/mL were purchased from Sigma-Aldrich, St. Louis, MO, USA.

2.2. Biofilm Growth and Staining Conditions

For biofilm assays, a PA14 overnight culture incubated at 37˚C under aerobic conditions in a rich medium (TSB) was diluted 1:100 into M63 minimal medium [18]. M63 medium was prepared as a 5X M63 stock solution by dissolving 15 g KH_2PO_4, 35 g K_2HPO_4, and 10 g $(NH_4)_2SO_4$ in 1 L of water, and diluting the 5X stock solution 1:5. M63 was supplemented with magnesium sulfate (1 mM), glucose (0.2%), and casamino acids (0.5%). 100 µL of the diluted culture was transferred into each well of a 96-well Falcon 3911 microtiter plate (Becton Dickinson and Company, Sparks, MD, USA). The microtiter plate was incubated for 24 hours at 37˚C. After incubation, the plate was inverted, and the liquid was removed. The plate was rinsed with

water by gently submerging it in a small container of water. Water was removed and the rinsing process was repeated a second time. This removed unattached cells and media components and significantly lowered background staining. To stain, 125 µL of a 0.1% solution of Crystal Violet (CV) (Sigma-Aldrich, St. Louis, MO, USA) in water was added to each well of the microtiter plate. The microtiter plate was incubated at room temperature for 10 - 15 min. After staining, the plate was rinsed 3 - 4 times with water by submerging it in a container of water as outlined above and blotted vigorously on a stack of paper towels to remove excess cells and dye. The microtiter plate was inverted and dried for a few hours.

2.3. The Effect of Cannabinoids in Inhibiting the Growth of PA14 Biofilms

To assess the effects of CBD and CBG in inhibiting the growth of a new biofilm, varying concentrations of each cannabinoid, ranging from 6.25 µG/mL to 200 µG/mL, were mixed with PA14 in M63 media and incubated in a 96-well Falcon 3911 microtiter plate (Becton Dickinson and Company, Sparks, MD, USA) overnight. As a control, M63 and PA14 were combined without the cannabinoid. The biofilms were subsequently incubated for 24 hours, which has been determined as the optimal time for biofilm growth of PA14. To view the results qualitatively, 0.1% crystal violet was used to stain wells and observe ring formation. Absorbance measurements were taken by solubilizing CV in 30% acetic acid to measure results quantitatively. Absorbance readings were measured by pipetting the contents from each well into a new microtiter plate and placing the plate in a Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fisher Scientific) and reading at 550 nanometers. Quadruplicate experiments were performed.

2.4. The Effect of Cannabinoids in Eradicating PA14 Biofilms

To assess the effects of CBD and CBG in eradicating a preexisting biofilm, M63 media was mixed with PA 14 and incubated in a 96-well microtiter plate overnight. The contents of the inoculated wells were then gently aspirated and rinsed three times by carefully aspirating the contents of each well and replacing them with fresh M63. Each cannabinoid at the varying concentrations noted above was then mixed with PA14 in M63 and incubated overnight. Additional wells on the plate served as a control. The rationale for the choice of media and incubation time is outlined above. To view the results qualitatively, 0.1% crystal violet was used to stain wells and observe ring formation. Absorbance measurements were taken by solubilizing CV in 30% acetic acid to measure results quantitatively, as described above. Quadruplicate experiments were performed.

2.5. Effect of Two Cannabinoids on PA14 Biofilms

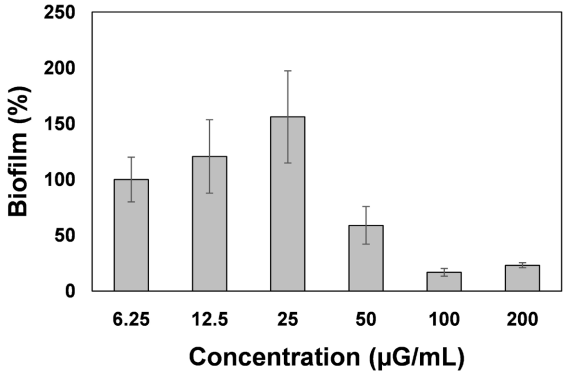
The effect of using CBD and CBG together was evaluated for both biofilm inhibition and eradication. The above procedures were performed as described above, but the concentrations of each cannabinoid were halved to maintain the overall

concentration of cannabinoids in the bacteria and M63 mixture.

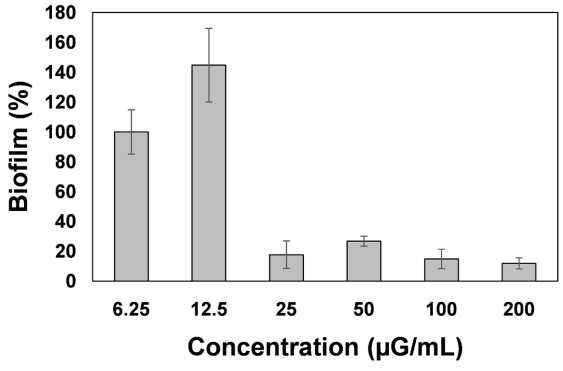
3. Results

3.1. Effect of CBD and CBG on the Inhibition of PA14 Biofilm Formation

Planktonic PA14 cells assayed by either a disk diffusion assay or an agar well-based assay did not result in any observable antimicrobial activity when treated with either CBD or CBG (data not shown). The ability of CBD and CBG to inhibit biofilm formation is presented in **Figure 1(A)** and **Figure 1(B)**, respectively. As shown, a greater level of inhibition is observed with CBG as compared to CBD. With CBG, 82.3% inhibition relative to control is observed with a concentration of 25 μG/mL. In contrast, CBD did not show inhibition at 25 μG/mL. With CBD, 84.6% inhibition was demonstrated with 100 μG/mL; with CBG, 85.02% inhibition was observed with 100 μG/mL. An initial increase in biofilm was observed at the lower concentrations of both CBD and CBG. This is consistent with results reported with *Streptococcus mutans* when CBD was added [7]. The enhancement of biofilm at low concentrations was also reported with the addition of antibiotics [19].



(A)

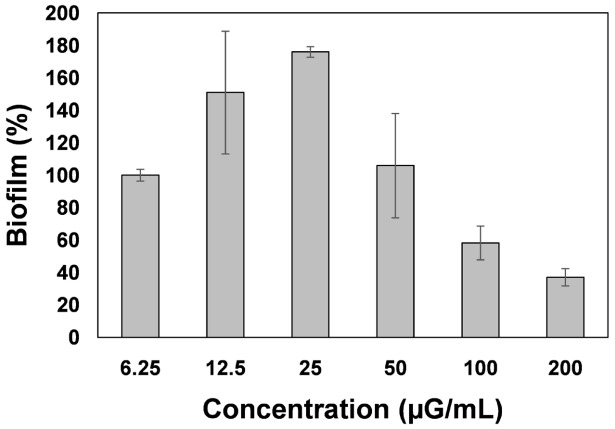


(B)

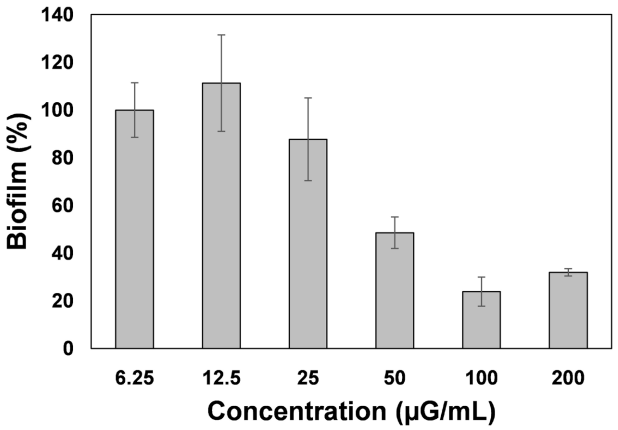
Figure 1. Biofilm inhibition assay. PA14 grown in the presence of (A) CBD and (B) CBG increasing in concentration (left to right) from 6.25 μG/mL to 200 μG/mL. Results represent the percent of biofilm measured relative to control (no cannabinoid addition) by a Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fisher Scientific) read at 550 nanometers. Quadruplicate experiments were performed.

3.2. Eradication of Preformed PA14 Biofilms by CBD or CBG

Varying concentrations of CBD or CBG, as described above in Methods, were applied to mature PA14 biofilms 24 hours after inoculation. Figure 2(A) and Figure 2(B) demonstrate the eradication of mature PA14 biofilms treated with different doses of CBD (Figure 2(A)) or CBG (Figure 2(B)). Eradication of PA14 biofilm was less efficient with CBD as compared to CBG. A concentration of 50 μG/mL showed no eradication with CBD. In contrast, 51.5% eradication was demonstrated with CBG. At 100 μG/mL, 42% eradication was demonstrated with CBD, whereas CBG resulted in 76.2% eradication. The inhibition and eradication experiments show an apparent differential activity between CBD and CBG, with CBG being more active.



(A)



(B)

Figure 2. Biofilm eradication assay. PA14 biofilms treated with (A) CBD and (B) CBG increasing in concentration (left to right) from 6.25 to 200 μG/mL. Results represent the percent of biofilm measured relative to control (no cannabinoid addition) by a Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fisher Scientific) read at 550 nanometers. Quadruplicate experiments were performed.

Evaluation of CBD and CBG in combination on PA14 biofilms. Because of the higher level of activity observed with CBG as compared to CBD in both the inhibition and eradication experiments, combinations of CBD and CBG were as-

sayed at the same dose level as tested in the individual inhibition and eradication experiments to investigate the effect of the combinations on PA14 inhibition and eradication. The objective was to determine whether CBG in combination with CBD could result in similar inhibition and eradication levels as observed with CBG alone. The inhibition of PA14 biofilm formation with combined CBD-CBG doses is shown in Figure 3. Eradication of a mature PA14 biofilm with combined CBD-CBG doses is represented in Figure 4. The results demonstrate that 90.6% inhibition with combined CBD-CBG doses, at 100 μG/mL (Figure 3), was superior relative to 85.09% eradication obtained with combined CBD-CBG doses (Figure 4). Interestingly, when combined with CBD, CBG drives greater inhibition at 100 μG/mL, 90.6% as compared to 84.6% with CBD alone. Similarly, combined CBD and CBG result in greater eradication, 85.09% as compared to 42% with CBD alone. The results demonstrate that CBG is more efficient in the inhibition and eradication of PA14 biofilms than CBD.

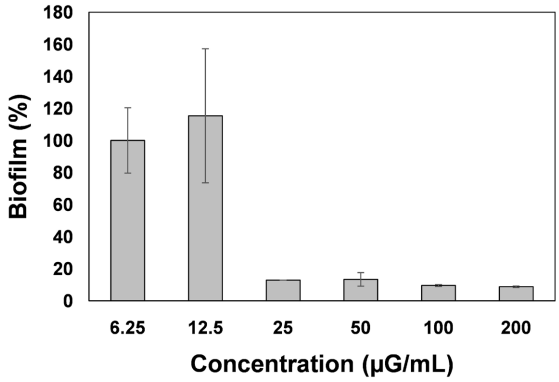


Figure 3. Inhibition assay with combined CBD and CBG doses. PA14 grown in the presence of CBD-CBG doses increasing in concentration (left to right) from 6.25 μg/mL to 200 μg/mL. Results represent the percent of biofilm measured relative to control (no cannabinoid addition) by a Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fisher Scientific) read at 550 nanometers. Quadruplicate experiments were performed.

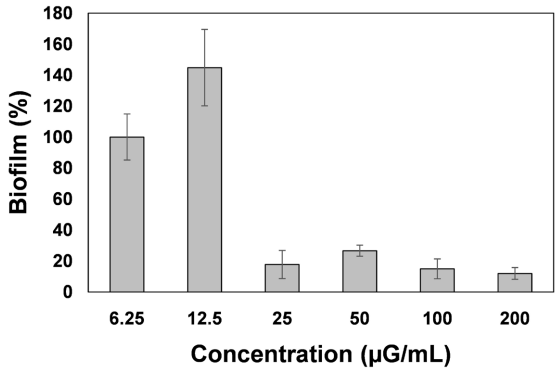


Figure 4. Eradication assay with combined CBD and CBG doses. PA14 biofilms were treated with CBD-CBG doses increasing in concentration (left to right) from 6.25 μG/mL to 200 μG/mL. Results represent the percent of biofilm measured relative to the control (no cannabinoid addition) by a Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fisher Scientific) read at 550 nanometers. Quadruplicate experiments were performed.

4. Discussion

Pseudomonas aeruginosa strain PA14, a burn wound isolate, is significantly more virulent than the routine laboratory strain PA01 [20]. Although PA14 is more virulent, its genome is highly conserved with that of PA01. Both PA01 and PA14 have been fully sequenced. The genome of PA01 encodes 5700 genes, and the genome of PA14 encodes 5892 genes [21]. PA14 harbors two pathogenicity islands which encode unique virulence-associated genes [22].

Treatment of patients infected with *P. aeruginosa* has been challenging; the bacterium is highly resistant to antibiotics. Resistance to antibiotics is more pronounced in biofilm-based infections [23]. The tendency of *P. aeruginosa* to cause persistent infections with increased mortality rates is attributed to its ability to form biofilms [24]. PA01 attaches quickly to a surface and progresses to biofilm formation in an irreversible process. In contrast, PA14 is slower at initial surface attachment, and biofilm formation is reversible. Because of its resistance to current therapeutics, the World Health Organization (WHO) has classified *P. aeruginosa* as one of the ESKAPE pathogens—pathogens in need of novel therapeutics [25]. As a consequence of the multidrug resistance in *P. aeruginosa*, the search for and discovery of novel therapeutics have been prioritized.

In this study, we show that CBD and CBG display dose-dependent antimicrobial activity in the inhibition and eradication of PA14 biofilms. For PA14, the antimicrobial activity is specific for biofilms. Planktonic PA14 cells assayed by either a disk diffusion assay or an agar well-based assay did not result in any observable antimicrobial activity when treated with either CBD or CBG (data not shown). Anti-biofilm activity is observed when CBD or CBG is used alone, or when the two cannabinoids are used together. Inhibition experiments evaluating each cannabinoid independently demonstrated greater sensitivity with CBG as compared to CBD. Similarly, experiments with combined CBD-CBG doses demonstrated greater sensitivity with inhibition than eradication. In contrast to most other studies that have evaluated the efficacy of cannabinoids as antimicrobial agents by focusing on either CBD or CBG, this study compared PA14 biofilm inhibition and eradication using both cannabinoids, CBD and CBG. Moreover, an additional uniqueness of this study entailed combinations of CBD and CBG evaluated for inhibition and eradication. Other studies reported in the literature have investigated combining a cannabinoid with either an antibiotic [16] or an antiseptic compound [26] [27] to enhance activity. This study showed enhanced activity of CBD in both inhibition and eradication assays when combined with a second cannabinoid, CBG. An initial increase in biofilm was observed at the lower concentrations of both CBD and CBG. This suggests a potential risk at sub-inhibitory concentrations. The increase in biofilm observed in our study is consistent with results reported with *Streptococcus mutans* when CBD was added [7]. The enhancement of biofilm at low concentrations was also reported with the addition of antibiotics [19]. The results of this *in vitro* study suggest that these compounds may be a viable option for individuals suffering from a biofilm infection. Addi-

tional validation in more complex systems including *in vivo* models or co-culture systems will be the focus of future studies.

Zeng *et al.* proposed that the anti-microbial mechanism of CBD against Gram-positive bacteria entails damage to the bacterial cell wall and cell membrane. Significant changes were observed in the proteomic and metabolic profiles. Processes affected include DNA binding, protein translation and expression, and phosphorylation [28]. Similarly, Blaskovich *et al.* demonstrated that protein, DNA, RNA, and peptidoglycan synthesis were all significantly inhibited when CBD was added to *S. aureus* [29]. Whether the anti-microbial mechanism of cannabinoids on *P. aeruginosa,* a Gram-negative organism, is comparable to the Gram-positive organisms or perhaps elicits changes in quorum sensing [30] will be the focus of future studies.

As resistance to antibiotic therapies continues to increase, the necessity for effective drug therapy becomes even more pronounced. Treatment options for *Pseudomonas aeruginosa,* a pathogen that manifests virulence by forming biofilms that are unresponsive to conventional antibiotics, remain limited. The findings from this study indicate that CBD and CBG exhibit anti-biofilm activity against this difficult pathogen. Because this study examined the effects of cannabinoids on a clinical isolate, the results are more reflective of what would be observed in actual patients. The implications of these discoveries have immense potential, as these compounds could be the future of treating stubborn biofilm infections in COPD exacerbations as well as other infections.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Short, B., Delaney, C., Johnston, W., Litherland, G.J., Lockhart, J.C., Williams, C., *et al.* (2024) Informed Development of a Multi-Species Biofilm in Chronic Obstructive Pulmonary Disease. *APMIS*, **132**, 336-347. https://doi.org/10.1111/apm.13386

[2] Nadell, C.D., Xavier, J.B. and Foster, K.R. (2009) The Sociobiology of Biofilms. *FEMS Microbiology Reviews*, **33**, 206-224. https://doi.org/10.1111/j.1574-6976.2008.00150.x

[3] Hall-Stoodley, L. and Stoodley, P. (2002) Developmental Regulation of Microbial Biofilms. *Current Opinion in Biotechnology*, **13**, 228-233. https://doi.org/10.1016/s0958-1669(02)00318-x

[4] Pragman, A.A., Berger, J.P. and Williams, B.J. (2016) Understanding Persistent Bacterial Lung Infections. *Clinical Pulmonary Medicine*, **23**, 57-66. https://doi.org/10.1097/cpm.0000000000000108

[5] Global Initiative for Chronic Obstructive Lung Disease (GOLD) (2022) Global Strategy for Diagnosis, Management and Prevention of COPD. 2022 Report. GOLD.

[6] Klahn, P. (2020) Cannabinoids-Promising Antimicrobial Drugs or Intoxicants with Benefits? *Antibiotics*, **9**, Article 297. https://doi.org/10.3390/antibiotics9060297

[7] Barak, T., Sharon, E., Steinberg, D., Feldman, M., Sionov, R.V. and Shalish, M. (2022) Anti-Bacterial Effect of Cannabidiol against the Cariogenic Streptococcus Mutans

Bacterium: An *in Vitro* Study. *International Journal of Molecular Sciences*, **23**, Article 15878. https://doi.org/10.3390/ijms232415878

[8] Kovacs, F.E., Knop, T., Urbanski, M.J., Freiman, I., Freiman, T.M., Feuerstein, T.J., *et al.* (2012) Exogenous and Endogenous Cannabinoids Suppress Inhibitory Neurotransmission in the Human Neocortex. *Neuropsychopharmacology*, **37**, 1104-1114. https://doi.org/10.1038/npp.2011.262

[9] Śledziński, P., Nowak-Terpiłowska, A. and Zeyland, J. (2020) Cannabinoids in Medicine: Cancer, Immunity, and Microbial Diseases. *International Journal of Molecular Sciences*, **22**, Article 263. https://doi.org/10.3390/ijms22010263

[10] Luz-Veiga, M., Amorim, M., Pinto-Ribeiro, I., Oliveira, A.L.S., Silva, S., Pimentel, L.L., *et al.* (2023) Cannabidiol and Cannabigerol Exert Antimicrobial Activity without Compromising Skin Microbiota. *International Journal of Molecular Sciences*, **24**, Article 2389. https://doi.org/10.3390/ijms24032389

[11] Di Onofrio, V., Gesuele, R., Maione, A., Liguori, G., Liguori, R., Guida, M., *et al.* (2019) Prevention of Pseudomonas Aeruginosa Biofilm Formation on Soft Contact Lenses by Allium Sativum Fermented Extract (BGE) and Cannabinol Oil Extract (CBD). *Antibiotics*, **8**, Article 258. https://doi.org/10.3390/antibiotics8040258

[12] Gildea, L., Ayariga, J.A., Ajayi, O.S., Xu, J., Villafane, R. and Samuel-Foo, M. (2022) Cannabis Sativa CBD Extract Shows Promising Antibacterial Activity against Salmonella Typhimurium and S. Newington. *Molecules*, **27**, Article 2669. https://doi.org/10.3390/molecules27092669

[13] Feldman, M., Sionov, R.V., Mechoulam, R. and Steinberg, D. (2021) Anti-Biofilm Activity of Cannabidiol against Candida Albicans. *Microorganisms*, **9**, Article 441. https://doi.org/10.3390/microorganisms9020441

[14] Aqawi, M., Sionov, R.V., Gallily, R., Friedman, M. and Steinberg, D. (2021) Anti-biofilm Activity of Cannabigerol against Streptococcus Mutans. *Microorganisms*, **9**, Article 2031. https://doi.org/10.3390/microorganisms9102031

[15] Aqawi, M., Gallily, R., Sionov, R.V., Zaks, B., Friedman, M. and Steinberg, D. (2020) Cannabigerol Prevents Quorum Sensing and Biofilm Formation of Vibrio Harveyi. *Frontiers in Microbiology*, **11**, Article ID: 858. https://doi.org/10.3389/fmicb.2020.00858

[16] Abichabki, N., Zacharias, L.V., Moreira, N.C., Bellissimo-Rodrigues, F., Moreira, F.L., Benzi, J.R.L., *et al.* (2022) Potential Cannabidiol (CBD) Repurposing as Antibacterial and Promising Therapy of CBD Plus Polymyxin B (PB) against Pb-Resistant Gram-Negative Bacilli. *Scientific Reports*, **12**, Article No. 6454. https://doi.org/10.1038/s41598-022-10393-8

[17] Maurice, N.M., Bedi, B. and Sadikot, R.T. (2018) *Pseudomonas aeruginosa* Biofilms: Host Response and Clinical Implications in Lung Infections. *American Journal of Respiratory Cell and Molecular Biology*, **58**, 428-439. https://doi.org/10.1165/rcmb.2017-0321tr

[18] O'Toole, G.A. (2011) Microtiter Dish Biofilm Formation Assay. *Journal of Visualized Experiments*, No. 47, Article No. 2437. https://doi.org/10.3791/2437-v

[19] Kaplan, J.B. (2011) Antibiotic-Induced Biofilm Formation. *The International Journal of Artificial Organs*, **34**, 737-751. https://doi.org/10.5301/ijao.5000027

[20] Mathee, K. (2018) Forensic Investigation into the Origin of Pseudomonas Aeruginosa PA14—Old but Not Lost. *Journal of Medical Microbiology*, **67**, 1019-1021. https://doi.org/10.1099/jmm.0.000778

[21] Ozer, E.A., Allen, J.P. and Hauser, A.R. (2014) Characterization of the Core and Ac-

cessory Genomes of Pseudomonas Aeruginosa Using Bioinformatic Tools Spine and Agent. *BMC Genomics*, **15**, Article 737. https://doi.org/10.1186/1471-2164-15-737

[22] Mikkelsen, H., McMullan, R. and Filloux, A. (2011) The Pseudomonas Aeruginosa Reference Strain PA14 Displays Increased Virulence Due to a Mutation in Lads. *PLOS ONE*, **6**, e29113. https://doi.org/10.1371/journal.pone.0029113

[23] Ciofu, O. and Tolker-Nielsen, T. (2019) Tolerance and Resistance of Pseudomonas Aeruginosa Biofilms to Antimicrobial Agents—How P. Aeruginosa Can Escape Antibiotics. *Frontiers in Microbiology*, **10**, Article ID: 913. https://doi.org/10.3389/fmicb.2019.00913

[24] Thi, M.T.T., Wibowo, D. and Rehm, B.H.A. (2020) Pseudomonas aeruginosa Biofilms. *International Journal of Molecular Sciences*, **21**, Article 8671. https://doi.org/10.3390/ijms21228671

[25] De Oliveira, D.M.P., Forde, B.M., Kidd, T.J., Harris, P.N.A., Schembri, M.A., Beatson, S.A., *et al.* (2020) Antimicrobial Resistance in ESKAPE Pathogens. *Clinical Microbiology Reviews*, **33**, e00181-19. https://doi.org/10.1128/cmr.00181-19

[26] Feldman, M., Gati, I., Sionov, R.V., Sahar-Helft, S., Friedman, M. and Steinberg, D. (2022) Potential Combinatory Effect of Cannabidiol and Triclosan Incorporated into Sustained Release Delivery System against Oral Candidiasis. *Pharmaceutics*, **14**, Article 1624. https://doi.org/10.3390/pharmaceutics14081624

[27] Avraham, M., Steinberg, D., Barak, T., Shalish, M., Feldman, M. and Sionov, R.V. (2023) Improved Anti-Biofilm Effect against the Oral Cariogenic Streptococcus Mutans by Combined Triclosan/CBD Treatment. *Biomedicines*, **11**, Article 521. https://doi.org/10.3390/biomedicines11020521

[28] Zeng, H., Wang, X., Tang, J., Liu, P., Zhang, S., Chu, H., *et al.* (2025) Proteomic and Metabolomic Analyses Reveal the Antibacterial Mechanism of Cannabidiol against Gram-Positive Bacteria. *Journal of Proteomics*, **315**, Article 105411. https://doi.org/10.1016/j.jprot.2025.105411

[29] Blaskovich, M.A.T., Kavanagh, A.M., Elliott, A.G., Zhang, B., Ramu, S., Amado, M., *et al.* (2021) The antimicrobial potential of cannabidiol. *Communications Biology*, **4**, Article No. 7. https://doi.org/10.1038/s42003-020-01530-y

[30] Berger, I., Kagan, A., Bock, R. and Loewy, Z.G. (2023) Chronic Obstructive Pulmonary Disease: Biofilm Mediated Exacerbation and Innovative Therapeutic Approaches. *EMJ Respiratory*, **11**, 99-107. https://doi.org/10.33590/emjrespir/10305099