Annales Universitatis Paedagogicae Cracoviensis
Studia Naturae, 8: 53–67, 2023, ISSN 2543–8832
DOI: 10.24917/25438832.8.3

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The effect of rose bengal activated with green diode laser light on selected Gram-positive and Gram-negative bacterial strains

Introduction

Pathogenic bacterial strains show increased resistance to antibiotic therapy threatening human health, thus other approaches to controlling bacterial infections are required. Antimicrobial photodynamic therapy (aPDT) is a promising alternative approach for treating infections that are difficult to treat with antibiotics. Its main advantage is that it does not lead to selection of resistant strains (Cieplik et al., 2018; Ghorbani et al., 2018). APDT involves application of a photoactivatable agent (a photosensitiser), for example rose bengal, prior to activation by light of an appropriate wavelength. The singlet oxygen species and/or free radicals generated upon illumination of this agent are toxic to pathogens (Sharma et al., 1999). The photodynamic effect may hit different targets, such as tumour tissues (Bonnett, 2002; Dolmans et al., 2003; Gunaydin et al., 2021), bacteria (Hamblin, Hasan, 2004; Liu et al., 2015; Martins Antunes de Melo et al., 2021), viruses and parasites (Fonseca et al., 2018; Varzandeh et al., 2021; Vital-Fujii, Baptista, 2021).

Rose bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein) is a stain belonging to xanthenes and is an analogue of fluorescein. Rose bengal (RB) was originally synthesised in 1882 by Swiss chemist Robert Ghnem (Alexander, 2010). RB sodium salt is commonly used in eye drops to stain damaged conjunctival and corneal cells and the first use of this pigment in ophthalmology dates back to 1914. In 1971, I RB (Robengatope®, rose bengal sodium I injection USP) was approved by the U.S. Food and Drug Administration (FDA) for use as a diagnostic agent in determining liver function (Baroyan, 1985; Gilger, Wilkie, 2013; Kurosu et al., 2022; Mincev, Zaharieva, 1974). Rose bengal dye has also been clinically investigated for the treatment of melanoma and other solid cancers (Kim, Rubio, 2011; Kurosu et al., 2022; Liu, Innamarato, 2016; Maker, Prabhakar, 2015; Patel et al., 2020; Qin, Kunda, 2017; Dhaini et al., 2023). According to Kurosu et al. (2022), the antibacterial activity of this dye strongly depends

on the purity of the compound used. They proved that a pharmaceutical grade RB was very effective in killing most Gram-positive bacteria under illumination conditions. Dahl et al. (1988) compared some species of Gram-positive and Gram-negative bacteria under illumination with RB and showed more sensitivity of Gram-positive species to photodynamic effect.

In this paper we describe the results of our research into the effect of aPDT in the presence of RB as a photosensitiser on selected strain of pathogenic bacteria which often show resistance to antibiotic therapy. One of such bacteria is *Staphylococcus aureus*. *S. aureus* is a Gram-positive bacterium that causes a wide variety of clinical diseases difficult to be treated (Boucher, Corey, 2008; *Centres for Disease Control and Prevention*, 2003; Taylor, Unakal, 2023). *S. aureus* is present in the environment as well as in normal human flora, located on the skin and mucous membranes of most healthy people. *S. aureus* does not normally cause infection on healthy skin, but in the bloodstream or internal tissues these bacteria may lead to serious infections (Lowy, 1998; Taylor, Unakal, 2023). The variability of *S. aureus* and the rapid adaptive response to changes in the environment and the resistance to antibiotics, cause the development of other alternative therapies. One of them is photodynamic therapy (PDT), which represents a promising approach to address the important shortage of new active antibiotics against multidrug-resistant *S. aureus* (Pérez et al., 2021).

Other bacteria species common in hospital environment and usually resistant to chemotherapeutics (e.g. quinolones) and antibiotics (e.g. vancomycin) is *Enterococcus faecalis*. *E. faecalis* (*E. faecium*) are Gram-positive commensal bacteria inhabiting gastrointestinal tracts of human. As an opportunistic pathogen, *E. faecalis* can cause life-threatening infections such as endocarditis, sepsis, urinary tract infections (UTIs) and root canal-treated teeth infections. Tennert et al. used successfully PDT to kill *E. faecalis* in experimental endodontic infections (2014). Recently, PDT turned out to be an alternative in the removal of oral biofilms and the prevention of oral cavity infections (López-Jiménez et al., 2015).

Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are also reservoirs of antibiotic resistance genes in hospital milieu. *E. coli* is Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium. Most of strains of *E. coli* are not pathogenic, naturally living in the gut. Virulent strains may cause gastroenteritis, urinary tract infections, haemorrhagic colitis, Crohn's disease, travellers' diarrhoea. Currently, the antibiotics of choice are fluoroquinolones or azithromycin and rifaximin. On the base of literature PDT effect was checked on the viability of *E. coli* cells (Garcez et al., 2020).

K. pneumoniae is a Gram-negative, rod-shaped non-motile, encapsulated facultative anaerobic bacterium. *Klebsiella* can cause the range of clinical diseases including pneumonia, thrombophlebitis, urinary tract infection, cholecystitis, diarrhoea, upper

respiratory tract infection, wound infection, osteomyelitis, bacteraemia, and sepsis. Nowadays, particularly dangerous multidrug-resistance bacterial strains (MDR) of *K. pneumoniae* spread into the environment and drinking water. *Klebsiella* possesses the resistance to ampicillin, many strains have acquired an additional resistance to carbenicillin, amoxicillin, and ceftazidime (Sarowska et al., 2022). Khan et al. (2017) examined the influence of methylene blue as a photosensitiser against *K. pneumonia* cultures *in vitro*. Eduardo et al. used this photosensitiser in vivo in oral mucosa (2019). Muskovic et al. (2023) examined the photodynamic inactivation (PDI) response using an exogenous photosensitiser cationic porphyrin with violet-blue light.

P. aeruginosa is a Gram-negative, aerobic-facultatively anaerobic, rod-shaped, encapsulated bacterium that can cause disease in humans (Diggle, Whiteley, 2020). *P. aeruginosa* causes infection in the urinary tract, respiratory system, dermis, soft tissue, bacteraemia, bone and joint, gastrointestine and blood, particularly in patients with severe burns, tuberculosis, cancer and AIDS. *Pseudomonas* in nature can exist in biofilm formats, attached to some surface or substrate, or in a planktonic form, as a unicellular organism, actively swimming using its flagellum (Driscoll et al., 2007). *P. aeruginosa* is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics. *P. aeruginosa* is a multidrug resistant pathogen. Mechanisms underlying to antibiotic resistance have been found to include production of antibiotic-degrading or antibiotic-inactivating enzymes, multidrug efflux pumps, mutations to change antibiotic targets and the low permeability of the bacterial cellular envelopes. Due to widespread resistance to many first-line antibiotics, carbapenems, polymyxins, and more recently tigecycline were considered to be the drugs of choice; however, resistance to these drugs has also been reported (Diggle, Whiteley, 2020). Therefore, research for the discovery of new strategies to eradicate *P. aeruginosa* are very much desired. Donnelly et al. (2007) reported that PACT Photodynamic antimicrobial chemotherapy (PACT) could be a potential alternative antimicrobial method in the treatment of *P. aeruginosa*.

In recent years the photodynamic activity of bengal rose activated with blue or green light against selected bacterial strains and fungi has been reported (de Oliveira Silva et al., 2023; Hung et al., 2022; Kitanaka et al., 2020; Wang et al., 2021). However, according to our knowledge, the differences between the sensitivity of Gram-positive and Gram-negative bacterial strains in the presence of this photosensitiser activated with green light have not described. Therefore, we decided to conduct research into the photodynamic effect with RB on a series of both Gram-positive (*S. aureus*, *E. faecalis*) and Gram-negative (*E. coli*, *K. pneumoniae* and *P. aeruginosa*) bacterial strains in order to check their sensitivity and conclude about the possibility of using RB as an effective photosensitiser in aPDT.

Materials and methods

The following bacterial strains from the American Type Culture Collection were tested: *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883 and *Staphylococcus aureus* ATCC 25923. Standard bacterial strains were cultured on LB medium for 24 hours at 37oC. After incubation of the plates the bacteria were placed in a densitometric tube containing 5 ml of sterile saline solution. The optical density of the bacterial suspension was determined using densitometer DEN-1&DEN1B (Grandbioinstument, Cambrige, UK) and adjusted to 0.5 on the McFarland scale (approximately 1.5 × 10^8 CFU/ml cells in the suspension).

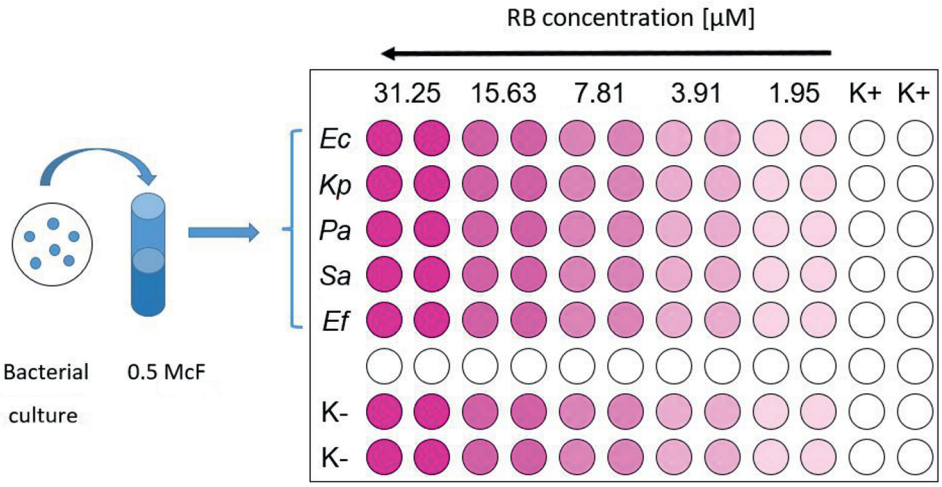

Fig. 1. Illustration of microtiter plate for showing the effect of RB on bacteria growth

Rose bengal (RB) was purchased in Acros Organics. Solutions of RB in 0.9% sterile saline were prepared at the following concentrations: 31.25 µM, 15.62 µM, 7.81 µM, 3.91 µM and 1.95 µM. The concentrations used were selected based on the literature (Dahl et al., 1988; Paulino et al., 2005; Nakonechny et al., 2019) and modified based on preliminary spectrophotometric measurements (data not included).

100 µL of liquid LB medium was placed into each of 96 wells of a microtiter plate, and then portions of 100 µL of RB solutions were applied to columns 1 to 10 and subsequently 10 µL of the appropriate bacterial suspension of the tested strains was added to each well. Columns K+ were positive controls for each bacterial strain, without the addition of RB, whereas rows K- were negative controls (without bacterial suspension) (Fig. 1). The prepared plates were irradiated with green diode laser light (λ = 535 nm,

P = 50 mW, t = 30 min and t = 45 min). A non-irradiated plate was the control. Next, the tested plates and the control plates were incubated in a thermoshaker (PST-60HL-4, Biosan, Poland) at 37°C for 20 hours. Turbidity measurements were carried out using a spectrophotometer Epoch (BioTech, Winooski, VT, USA) at a wavelength of 570 nm.

Results and short discussion

The results of our study demonstrate aPDT using green laser light and rose bengal on selected standard strains of *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Mean turbidance values and Standard Deviations for each tested bacteria species irradiated with green light for 30 min, 45 min and the control group in dark conditions in the presence of RB solutions are presented in tables (1–6).

Irradiation in the presence of RB in all tested solutions reduces the growth of *S. aureus*. MBC equals 1.95 μM. In the darkness RB solutions reduce bacterial growth at the higher concentrations and MBC equals 15.6 μM (Tab. 1).

Tab. 1. Mean ± Standard Deviation turbidance values for *Staphylococcus aureus* irradiated with diode laser light for 30 min, 45 min and the control group in dark conditions in the presence of RB solutions

RB[μM] Conditions	31.25	15.63	7.81	3.91	1.95	0
Light 30 min	0.221 ± 0.005	0.133 ± 0.003	0.089 ± 0.002	0.068 ± 0.001	0.058 ± 0.002	0.629 ± 0.031
Light 45 min	0.223 ± 0.028	0.127 ± 0.006	0.083 ± 0.003	0.063 ± 0.003	0.055 ± 0.001	0.697 ± 0.052
Dark	0.231 ± 0.009	0.141 ± 0.004	0.578 ± 0.065	0.513 ± 0.054	0.547 ± 0.037	0.568 ± 0.039

Pérez et al. (2021) demonstrated the efficiency of photodynamic therapy in eliminating *S. aureus* strains. Dadras et al. (2006) used green light at 514 nm to eliminate these bacteria strains. Kim et al. (2013) reported that green light was especially effective against *S. aureus* and these bacteria were killed only by light 525 nm. Their results suggest that bacteria membrane structure is an important factor in the bactericidal effect of light therapy.

After 30- and 45-min irradiation, the reduction of bacterial optical density is observed in case of *E. faecalis*. MBC value equals 15.6 μM. At lower RB concentrations, after irradiation the bacteriostatic effect is observed (MIC=1.95 μM), whereas in the darkness only 7.8 μM RB solution decreases turbidance value to about 50% of positive control group. MIC value of RB in the darkness equals 7.8 μM, whereas MBC value 15.6 μM. The obtained results show that green light irradiation inhibits the growth of *E. faecalis* (Tab. 2).

Tab. 2. Mean ± Standard Deviation turbidance values for *Enterococcus faecalis* irradiated with diode laser light for 30 min, 45 min and the control group in dark conditions in the presence of RB solutions

RB [μM] Conditions	31.25	15.63	7.81	3.91	1.95	0
Light 30 min	0.218 ± 0.010	0.134 ± 0.004	0.288 ± 0.004	0.266 ± 0.001	0.337 ± 0.022	0.506 ± 0.063
Light 45 min	0.214 ± 0.006	0.127 ± 0.006	0.211 ± 0.100	0.263 ± 0.081	0.248 ± 0.024	0.653 ± 0.054
Dark	0.212 ± 0.016	0.157 ± 0.035	0.314 ± 0.041	0.770 ± 0.092	0.801 ± 0.109	0.734 ± 0.083

Dahl et al. (1988) demonstrated that the Gram-positive species, *Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis* and *Streptococcus salivarius,* were inactivated about 200x more quickly (99% inactivation) than Gram-negative *Salmonella typhimurium* wildtype strain. Their results suggest that the inability of Gram-positive bacteria to exclude the anionic photosensitiser, rose bengal, may account for a greater degree of inactivation of these cells compared to Gram-negative bacteria. Penetration of RB into cells has been deemed essential for the photodynamic action (Ito, 1980). We can conclude that RB penetrates the membrane of *E. faecalis*, as we observe the decrease of cell growth in the darkness at RB higher concentrations, whereas green light illumination enhances the effect of inhibition because of PDT reactions.

After 45 min irradiation weak inhibition is observed and MIC value is 3.9 μM, whereas MBC equals 31.25 μM. Shorter time of irradiation slightly inhibits the growth of E. coli and MIC equals 15.6 μM. RB does not reveal antibacterial activity even in the darkness (Tab. 3).

Tab. 3. Mean ± Standard Deviation turbidance values for *Escherichia coli* irradiated with diode laser light for 30 min, 45 min and the control group in dark conditions in the presence of RB solutions

RB [μM] Conditions	31.25	15.63	7.81	3.91	1.95	0
Light 30 min	0.917 ± 0.028	0.825 ±0.021	0.843 ± 0.063	0.810 ± 0.043	0.767 ± 0.038	0.808 ± 0.005
Light 45 min	0.225 ± 0.043	0.735 ± 0.108	0.776 ± 0.066	0.666 ± 0.046	0.699 ± 0.055	0.795 ± 0.080
Dark	0.986 ± 0.036	0.853 ± 0.031	0.818 ± 0.034	0.794 ± 0.039	0.772 ± 0.033	0.748 ± 0.047

The lipopolysaccharide portion of the cell wall of Gram-negative bacteria serves as a barrier to a variety of potentially toxic substances (Dahl et al., 1988). This capacity almost certainly enhances the survival of these bacteria in the presence of RB in the darkness. We observe weak inhibition of the bacteria growth after irradiation because of inability

to penetrate the cell wall by RB in these experimental conditions, thus the illumination can activate RB but on the surface of the cell of *E. coli.*

No antibacterial effect is observed in the presence of RB both after irradiation and in dark conditions in case of *K. pneumoniae* (Tab. 4).

Tab. 4. Mean ± Standard Deviation turbidance values for *Klebsiella pneumoniae* irradiated with diode laser light for 30 min, 45 min and the control group in dark conditions in the presence of RB solutions

RB [μM] Conditions	31.25	15.63	7.81	3.91	1.95	0
Light 30 min	0.861 ± 0.116	0.732 ± 0.008	0.654 ± 0.060	0.690 ± 0.052	0.573 ± 0.048	0.639 ± 0.014
Light 45 min	0.942 ± 0.067	0.863 ± 0.031	0.788 ± 0.054	0.713 ± 0.028	0.710 ± 0.028	0.721 ± 0.033
Dark	0.954 ± 0.050	0.911 ± 0.024	0.875 ± 0.026	0.769 ± 0.029	0.728 ± 0.012	0.718 ± 0.014

Amadeo et al. (2022) reported that *K. pneumoniae* proved to be the resistant strain to a blue and violet blue light and the observed difference in sensitivity of various bacterial strains to light irradiation was associated with a different composition endogenous porphyrins. *K. pneumoniae* is insensitive to RB treatment due to the permeability barrier of the outer membrane. Gram-negative bacteria are often found to be significantly resistant to photosensitiser treatment (Malik et al., 1992). However, inactivation of Gram-negative bacteria was effective when the photosensitisers used contained a net cationic charge (Merchat et al., 1996; Minnock et al., 1996) or when the bacterial membrane was permeabilised by polymyxin B nonapeptide (PMBN) in the presence of a noncationic photosensitiser (Nitzan et al., 1992).

A bacteriostatic effect is observed after 30 min irradiation for *P. aeruginosa* and MIC value equals 31.25 μM. 45 min irradiation causes reduction of bacterial optical density in the presence of RB at all tested concentrations (MBC=1.95 μM) (Tab. 5).

Tab. 5. Mean ± Standard Deviation turbidance values for *Pseudomonas aeruginosa* irradiated with diode laser light for 30 min, 45 min and the control group in dark conditions in the presence of RB solutions

RB [μM] Conditions	31.25	15.63	7.81	3.91	1.95	0
Light 30 min	0.815 ± 0.125	1.135 ± 0.05	1.086 ± 0.04	0.997 ± 0.035	0.948 ± 0.077	0.950 ± 0.049
Light 45 min	0.225 ± 0.008	0.132 ± 0.005	0.084 ± 0.003	0.066 ± 0.005	0.053 ± 0.002	0.988 ± 0.057
Dark	1.105 ± 0.080	1.082 ± 0.107	1.066 ± 0.064	0.957 ± 0.093	0.969 ± 0.078	0.942 ± 0.075

Guffey and Wilborn (2006) examined the *in vitro* effects of 405 and 470 nm light on two common aerobes, *S. aureus* and *P. aeruginosa,* and reported different bactericidal effects depending upon wavelength. The killing rate of 405 nm light was greater than 470 nm light (90% for *S. aureus* and 95.1% for *P. aeruginosa*. Briggs et al. (2018) observed the effect of the PDT with methylene blue (MB) on *P. aeruginosa* biofilm. MB alone significantly reduced bacterial growth but did not enable complete eradication of *P. aeruginosa* biofilm. APDT effect was observed on a clinical isolate of P. *aeruginosa* strain *in vitro*. *In vivo* aPDT reduced bacterial load in burn wounds and delayed bacteremia (Hashimoto et al., 2012).

Table (6) illustrates the background, RB at different concentrations, in all tested samples. No significant differences dependently light conditions are observed.

Tab. 6. Mean ± Standard Deviation turbidance values for negative control (without bacteria) irradiated with diode laser light for 30 min, 45 min and the control group in dark conditions in the presence of RB solutions

RB [μM] Conditions	31.25	15.63	7.81	3.91	1.95	0
Light 30 min	0.212 ± 0.016	0.129 ± 0.003	0.089 ± 0.002	0.062 ± 0.003	0.056 ± 0.004	0.046 ± 0.002
Light 45 min	0.219 ± 0.004	0.125 ± 0.003	0.088 ± 0.000	0.062 ± 0.001	0.055 ± 0.003	0.045 ± 0.001
Dark	0.222 ± 0.010	0.135 ± 0.005	0.089 ± 0.006	0.066 ± 0.004	0.056 ± 0.003	0.045 ± 0.003

45 min of green diode laser light irradiation effectively inhibited the growth of *S. aureus*, *E. faecalis* and *P. aeruginosa* at each tested concentration. However, Gram-negative bacteria, *K. pneumonia* and *E. coli* were insensitive to irradiation of the system for 45 min regardless of RB concentration. Growth inhibition of *E. coli* was observed only in case of 31.25 μM RB.

Non-activated RB inhibited the growth of *S. aureus* and *E. faecalis* only at the two highest concentrations. 30 min irradiation inhibited the growth of *S. aureus* and *E. faecalis* at all tested RB concentrations. The growth of *P. aeruginosa* irradiated for 45 min was not inhibited (Fig. 2).

Conclusions

Rose bengal as a photosensitiser inhibited the growth of Gram-positive bacteria at the highest concentrations. *Staphylococcus aureus* and *Enterococcus faecalis* were the most photosensitive strains due to the structure of the bacterial cell wall and higher possibility of rose bengal penetration into the bacterial cell, leading to the bacteriostatic effect. Additionally, the light perception may be achieved by photoreceptor proteins. Some species of bacteria have evolved the capability to respond to a proper light wavelength

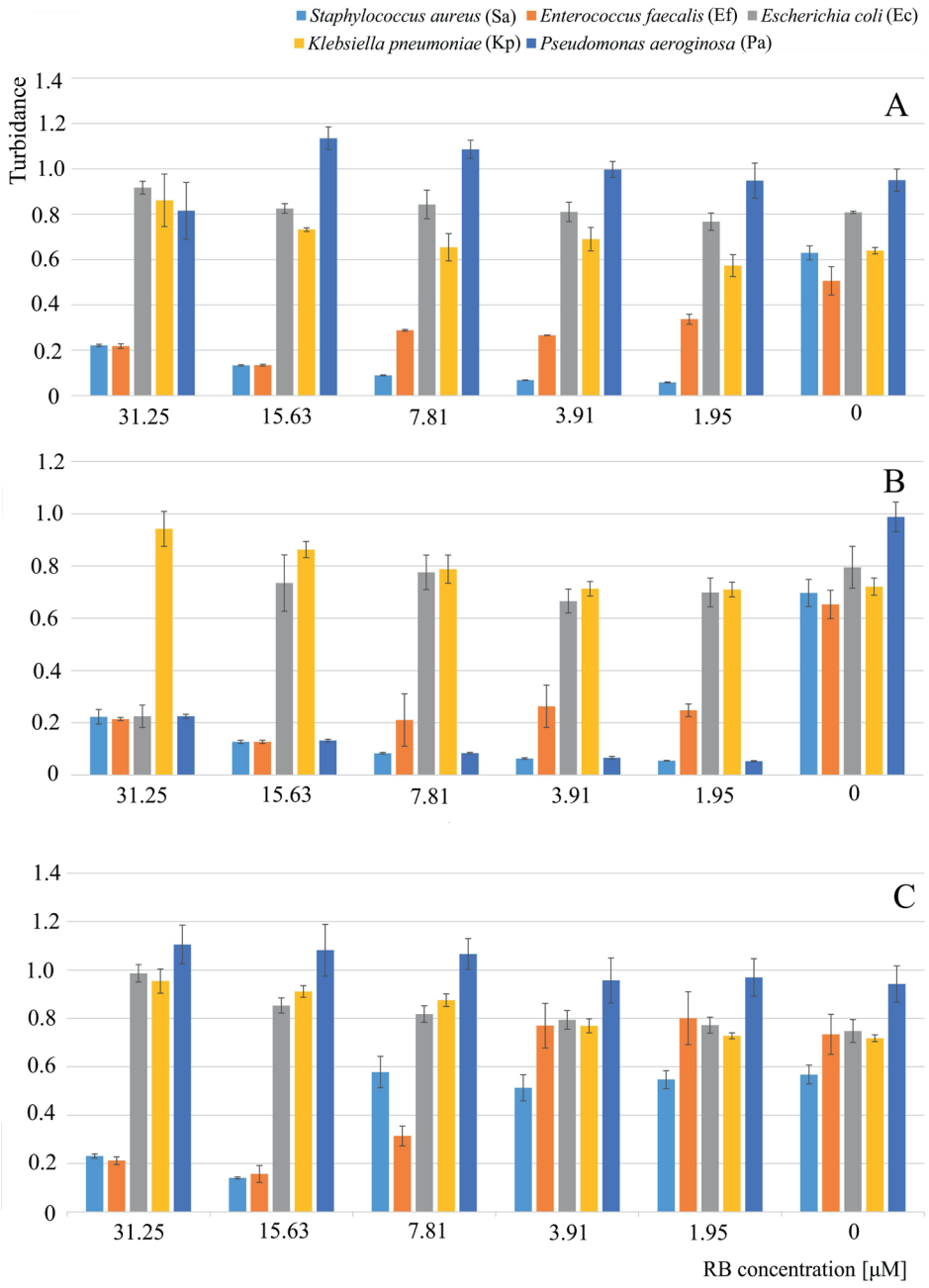

Fig. 2. The effect of irradiation with 535 nm diode laser light on the growth of different standard bacterial strains in the presence of RB solutions at different concentrations: A – 30 min., B – 45 min; the growth of different standard bacterial strains in the presence of RB solutions at different concentrations under dark conditions – C

(Kraiselburd et al., 2017). Growth inhibition of Gram-positive bacteria was observed after 30 min exposure. Longer time of irradiation (45 min) enhanced this effect. Gram-negative bacterial strains, *Klebsiella pneumoniae* and *Escherichia coli*, turned out to be the least sensitive to the photodynamic effect in the presence of rose bengal. The structure of the cell wall of Gram-negative bacteria hinders rose bengal penetration into the cell. Additionally, *Klebsiella pneumoniae* is characterised by the presence of a mucoid capsule, which makes the bacterial cell more resistant to aPDT effect.

On the basis of the obtained results and literature we conclude that photodynamic therapy is a promising approach of bacteria treatment, also multidrug resistant strains. Our work aims to confirm the significance of combining light, photosensitiser and oxygen in antibacterial therapy. In the future we plan to check the influence of antibiotics, a photosensitiser and light on drug resistant gram-positive bacteria.

Conflict of interest

The authors declare no conflict of interest related to this article.

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Abstract

In recent years the photodynamic activity of rose bengal activated with green light against selected bacterial strains has been reported. However, according to our knowledge, the differences between the sensitivity of Gram-positive and Gram-negative bacterial strains in the presence of this photosensitiser have not been described. The aim of the conducted research was to examine the antibacterial effect of 535 nm wavelength diode laser light in the presence of rose bengal as photosensitiser on selected reference bacterial strains: *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. Sterile 96-well microtiter plates were used to determine the antibacterial activity of the green light and rose bengal solutions at various concentrations. The labelled bacterial suspensions were placed to each well of the 96-well microtiter plate filled with liquid medium LB and solution of rose bengal. The plates were exposed to green diode laser light. After 24 hours of incubation at 37oC, the turbidance was read in a spectrophotometer. The irradiation in the presence of photosensitiser can act in an antibacterial manner, either bacteriostatically or bactericidally. The tested strains exhibit different sensitivity to irradiation because of the structure of the cell wall, the presence of different bacterial pigments and photoreceptor proteins in some species of bacteria. Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* were the most photosensitive strains due to the higher possibility of rose bengal penetration into the bacterial cell, leading to the bacteriostatic effect. Our results show that rose bengal may be applied in the treatment of Gram-positive infections.

Keywords: antimicrobial, diode laser light irradiation, photodynamic effect, rose bengal

Abbreviations: RB, – rose bengal, (a)PDT – (antimicrobial) photodynamic therapy, K+ – positive control (with bacteria), K- – negative control (without bacteria)

Received: [2023.10.06]

Accepted: [2023.12.20]

Wpływ różu bengalskiego aktywowanego światłem lasera zielonej diody na wybrane szczepy bakterii Gram-dodatnich i Gram-ujemnych

Streszczenie

W ostatnich latach pojawiły się doniesienia literaturowe o aktywności fotodynamicznej różu bengalskiego aktywowanego światłem zielonym względem wybranych szczepów bakterii. Jednakże, według naszej wiedzy, nie opisano dotychczas różnic we wrażliwości na ten aktywowany światłem zielonym fotosensybilizator pomiędzy bakteriami Gram-dodatnimi i Gram-ujemnymi. Celem przeprowadzonych badań było określenie przeciwbakteryjnego działania diodowego światła laserowego o długości fali 535 nm w obecności różu bengalskiego jako fotosensybilizatora na wybrane wzorcowe szczepy bakterii *Pseudomonas aeruginosa*, *Enterococcus faecalis, Escherichia coli*, *Klebsiella pneumoniae* oraz *Staphylococcus aureus*. W celu określenia aktywności przeciwbakteryjnej roztworów różu bengalskiego w różnych stężeniach, naświetlanego światłem, wykorzystano sterylne 96-dołkowe płytki mikrotitracyjne. Standaryzowane zawiesiny bakterii umieszczono w każdym dołku płytki mikrotitracyjnej wypełnionej płynną pożywką LB i roztworem różu bengalskiego. Płytki naświetlano światłem zielonym. Po 24 godzinach inkubacji w temperaturze 37°C odczytywano wartości turbidancji. Diodowe światło laserowe o długości fali 535 nm w obecności fotosensybilizatora może działać antybakteryjnie, bakteriostatycznie lub bakteriobójczo. Badane szczepy bakterii wykazują różną wrażliwość na działanie zielonego światła w obecności różu bengalskiego z powodu różnic w budowie ściany komórkowej, obecności różnych barwników bakteryjnych oraz fotoreceptorów u niektórych gatunków bakterii. Bakterie Gram-dodatnie, *Staphylococcus aureus* and *Enterococcus faecalis* były najbardziej foto-wrażliwe ze względu na wyższe prawdopodobieństwo wnikania różu bengalskiego do wnętrza komórki bakteryjnej, prowadzącego do efektu bakteriostatycznego. Nasze wyniki pokazują, że róż bengalski może zostać wykorzystany w leczeniu infekcji wywołanych bakteriami Gram-dodatnimi.

Słowa kluczowe: działanie przeciwdrobnoustrojowe, naświetlanie diodowym światłem laserowym, efekt fotodynamiczny, róż bengalski

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