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THE ACTIVITY OF SELECTED DISINFECTANTS TOWARDS ENDOSPORES OF BACTERIA OF THE *BACILLUS* GENUS

PAULINA OLESIAK[1], ALEKSANDRA SMYŁŁA[1], PIOTR KRUPA[1], MACIEJ KOSTECKI[2]

[1]Department of Microbiology and Biotechnology, Jan Dlugosz Academy,
A. Armii Krajowej 13/15, 42-200 Częstochowa, Poland
[2]Institute of Environmental Engineering of the Polish Academy of Sciences
Skłodowskiej-Curie 34, 41-819 Zabrze, Poland
Corresponding authors e-mail: paulina_olesiak@interia.eu; asmylla@gmail.com; kostecki@ipis.zabrze.pl

Abstract: Disinfectants are commonly used in households, hospitals, in drug manufacturing, in food processing. With the ever-increasing antibiotic-resistance of microorganisms, it is crucial to rationally apply disinfectants in suitable concentrations, with proper active substance, as not all substances affect various organisms in the same way. Among the microorganisms that are particularly difficult to kill, there are bacteria producing spores – forms that have different structure and sensitivity to disinfectants than the vegetative forms. The aim of the study has been to examine the influence of frequently used disinfectant compounds upon the spores of bacteria of the *Bacillus* genus: *B. cereus*, *B. mycoides*, and *B. subtilis*. In the study of disinfectants the findings showed that the disinfectants with the best results against spores are: peracetic acid, hydrogen peroxide in concentrations of 30% as well as 5%, and Lysoformin 3000. The least efficient in fighting spores proved to be Isopropanol and Promanum N. Differentiation has been found to exist in the reaction of specific species to the preparation Rafasept, as *B. subtilis* occurred to be very sensitive to that compound, whereas Rafasept turned out to be ineffective in the case of *B. mycoides* and *B. cereus*.

INTRODUCTION

Microorganisms existing in the air, dust, on the hands of staff, on shoes, hair, as well as equipment and tools [14, 15] constitute a continuous source of infections in large communities of patients, often with reduced natural immunity [16].

Prevention of those infections consists in interrupting the transmission paths and destroying their sources, by applying various means that have the task of killing or eliminating microorganisms [16]. Biocidal products, such as antiseptics, disinfectants, and preservatives, which have been in use in various forms for many years, play a vital role in infection control and prevention of transmission of microorganisms [14, 24].

In the majority of available disinfectants, the antibacterial action concerns vegetative forms of bacteria [14, 16, 19, 24]. A much greater challenge for disinfection is posed by the bacterial spores, which are characterized by higher resistance to all physical and chemical factors than the vegetative forms [14, 19].

The spore-producing bacteria, common in the environment, belonging to the *Bacillus* and *Clostridium* genus, are the cause of numerous problems in food processing industry, in pharmaceutical industry, as well as in medicine. Responsible for food poisoning are, among others: *Bacillus cereus*, *Clostridium botulinum* or *Clostridium perfringens* [9, 31, 32]. Bacteria of the Bacillus genus are encountered on the skin, sometimes they even dominate in skin swabs [28], also, due to improper disinfection or sterilization, they may, alongside with the *Pseudomonas* or *Serratia* rods, be the cause of epidemics of hospital infections [26].

In disinfection, depending on the required cleanliness class [26], three levels are distinguished: low (where the requirement is to destroy vegetative forms of bacteria and certain viruses); medium disinfection level (the requirement is to inactivate mycobacteria and viruses); and high level (disinfection should effectively destroy spores, fungi, and viruses) [22].

The effectiveness of disinfectants is connected, among other things, with diffusion of those compounds to cells [16]. Intracellular penetration of antibiotics and biocides depends upon the construction of bacteria cell walls, the presence of the external membrane, or generation of the spore external coat [25].

A distinct reaction of bacteria spores to disinfectants is related to their specific structure. The spore, containing dipicolinic acid, is surrounded by a system of cell coats (shields), which constitute as much as 50% of the dry mass of the entire spore [21, 31].

The outermost part is the exosporium, made of lipid-protein membrane, with carbohydrates present [31]. This is followed, in that order, by: external coat of the spore and internal coat of the spore made up mainly of proteins [21], as well as the cortex, which constitutes the thickest layer protecting the core, and is made of a distinct type of mucopeptide. The core of the spore is made of cytoplasm, surrounded by protein – a lipid membrane, containing proteins, DNA, RNA [21, 31]. The spore coat and cortex constitute a barrier preventing biocides from entering and penetrating the inside of the spore [23].

Differences in disinfectant activity are related also to the differences in their chemical structure, they depend mainly upon the so-called compound active group [12].

Among the various groups of active substances used in disinfection, the so-called reactive substances stand out in terms of effectiveness. They are strongly reactive chemically and they destroy microorganisms entering chemical reactions. These are: aldehydes, halogens and their compounds, and peroxides [12]. Among the disinfectant compounds that are active towards spores, one can distinguish compounds that inhibit the germination and growth of spores, the so-called sporostatic compounds, included by Russell in group A, and sporo-cidal compounds that render spores inactive, included in group B [21].

Among the sporocidal compounds of group B there are aldehyde, chlorium and iodine compounds, hydrogen peroxide, hydroxy-acids, ethylene oxide, and others [21]. Disinfectants based upon glutaraldehyde are effective, among other things, in inactivating *Bacillus anthracis* spores [13]. Glutaraldehyde has a broad spectrum of activity and is characterized by rapid action [29]. Glutaraldehyde forms strong cross-bonds with amino groups of the cell wall, and the spore bacterial coat [23].

The strength of the disinfectant depends also upon its concentration and action time [16]. Hydrogen peroxide, which is a strong disinfectant, proves to be effective towards spores only in high concentrations or at higher temperatures [12].

Organic peroxi-acids, such as per-acetic acid, have much better effectiveness [12]. Peracetic acid has a broad range of activity, affecting bacteria, spores, fungi, algae, and viruses [29]. It is supposed that hydroxy radicals which get formed cause irreversible damage to reduced functional groups of proteins, and thus destroy the enzymatic apparatus and cell structure. The latter leads, among other things, to leakiness of cell membranes [12].

The group of sporostatic compounds, inhibiting the development of spores, the so-called group A, consists of phenols, alcohols, organic acids, and others [21]. Alcohols are commonly used as ingredients of disinfectants used in hand washing, for hygienic purposes and scrubbing, for disinfecting skin, as well as surfaces and medical equipment. The most frequently used alcohols include ethanol, isopropanol, and n-propanol [10]. In the group of sporostatic compounds, one can also list products based on phenol, affecting bacteria cell membranes and causing coagulation of cytoplasm structures [23].

The aim of the study has been to compare the activity of frequently used disinfectant compounds having various functional groups, upon the spores of aerobic bacteria spores of *Bacillus cereus*, *Bacillus subtilis*, and *Bacillus mycoides* strains.

EXPERIMENTAL PROCEDURES

The investigation concerning the effects of selected disinfectants on spores was conducted on the following strains: *Bacillus subtilis* ATCC 6633, *Bacillus mycoides* ATCC 8896 and *Bacillus cereus* ATCC 128276. *B. subtilis* and *B. mycoides* were obtained from the University collection, while the strain *B. cereus* was obtained from the Collection of Microorganisms of the Polish Academy of Sciences in Wrocław (L. Hirszfeld Institute of Immunology and Experimental Therapy). To compare the efficacy of the tested disinfectants as concerns the spores of *Bacillus*, control studies were conducted on non-sporing bacteria. *Escherichia coli* PCM 2209 and *Pseudomonas aeruginosa* PCM 499 were selected for this purpose. The strains came from the university collection.

The following disinfectants were tested: Steridial P (based on peracetic acid), 5% and 30% Hydrogen peroxide, Lysoformin 3000 (based on glutaraldehyde), Rafasept (based on phenol), Promanum N (based on ethanol), and Isopropanol 98%.

To study the sensitivity of strains the following media were applied: TSB (Tryptic Soy Brouth): Tryptone 17.0; soya peptone 3.0; NaCl 5.0; K_2HPO_4 4.0 g/l; TSA (Tryptic Soy Agar): tryptone 15.0; soya peptone 5.0; NaCl 5.0; agar 15.0 g/l [18]; TSB with neutralizer: Tryptone 17.0; soya peptone 3.0; NaCl 5.0; K_2HPO_4 4.0; Tween 80 10.0; lecithin 1.0; histidine L 0.5; $Na_2S_2O_3$ 2.5 g/l [13, 18].

The following experimental procedure was used:
1. Preparation of the inoculum: the weekly culture of strains was rinsed with 10 ml of 0.85% NaCl solution, the suspension was centrifuged for 1 min. and then exposed to the temp. of 72°C for 10 minutes in water bath [oral information], [modified 18].
2. Course of the experiment:
   a) 1 ml of the spore suspension was transferred to 9 ml of the TSB medium (control sample), from which portions of 0.1 ml were cultured (spread onto 0.1 ml per dish) on TSA medium, in order to determine the number of spores in the suspension (cfu – colony forming units).
   b) 1 ml of spores suspension was transferred to 9 ml of each of the disinfectants tested. After 5, 10, 15, 30, 60, 120 and/or 180 minutes of exposure to the disinfectant, one

ml of each suspension was transferred to 9 ml of TSB medium with neutralizer, for 30 min.
c) the number of spores (cfu) was determined by culturing the samples on stable TSA medium, as a diluent the TSB medium was used [18].

The cultures were incubated for 24 hours in the temperature of 28°C in the case of *Bacillus*, and in 37°C in the case of *E. coli* and *Ps. aeruginosa*.

The above scheme was used for all strains and disinfectants [17, 18]. Results are presented on diagrams and given as log of colony forming units [cfu] in 1 ml, grown on TSA medium.

RESULTS AND DISCUSSION

The ever better recognition of the causes of nosocomial infections (hospital-acquired infections) is a reason for increased demand for disinfectants used to get rid of the microorganism reservoirs and to eliminate the paths through which infections spread [8].

Ineffectiveness of disinfectants, as regards spores, creates enormous problems in disinfection, in particular in such locations as hospitals, where sterile conditions are extremely important, as it pauses a threat to lives of patients, through opportunistic infections [15].

Application of unsuitable disinfectants or disinfectants in improper concentrations is conducive to the generation of forms that are resistant to such disinfectants [2]. The more so, in such locations as hospitals one often encounters cross-infections, which may result in even greater problems [16].

Peracetic acid, as one of the few compounds, affects in low concentrations all types of microorganisms: vegetative bacteria (gram positive and gram negative), spores, fungi, and viruses [6].

However, there are certain differences concerning the efficacy of definite concentrations. Matras and Bartoszcze state that effective disinfection of the environment infected with anthrax bacilli should be carried out by means of 3% peracetic acid [11]. Also in the study concerning the influence of 5% peracetic acid upon *B. anthracis* spores conducted by Mizak it has been demonstrated that those forms die after 120 minutes, whereas at the concentration reaching up to 3% the time amounted to 180 minutes [13]. Also, according to Jonem and Turnbull (quoted after Mizak [13]) the cidal effect of 3% and 5% peracetic acid upon the *B. anthracis* spores becomes evident after 120 and 180 minutes, respectively.

Somehow different values have been obtained by Hussaini and Ruby (quoted after Mizak [13]) in the study of effectiveness of 5% peracetic acid to deal with *B. anthracis*, there the total spore-cidal effect has already been demonstrated after 20 minutes. The same result has been obtained by Baldry and Manche (quoted after Mizak [13]). Perhaps pH level has significance, as the spore-cidal action of peracetic acid at the pH of 3 and concentration of 300 ppm requires a 30-minute exposure, whereas in the case of more alkaline pH effective decontamination requires an increased concentration, as well as longer exposure time [7].

According to Staniszewska *et al.* [27] various methods lead to various results, what is important is the selection of strains, media, and culture conditions, as in the research done using suspension method, the peracetic acid had cidal effects after 15–30 minutes, already in the concentration of 0.1%. However, in the investigation of a preparation con-

sisting of peracetic acid and hydrogen peroxide using carrier method, the time required for reduction of *B. subtilis* and *B. cereus* spores got extended to 11 hours [27]. Differentiation of the reactions of sporing bacteria to peracetic acid may depend upon strain-specific properties; Hilgren *et al.* [4] found that *B. cereus* spores are by 1.5–2.5 log more resistant to peracetic acid than the *B. subtilis* spores.

In the studies based on the suspension method, concerning the influence of Steridial P, whose main component is 1% peracetic acid, it has been demonstrated that spores of all three tested strains: *B. subtilis*, *B. mycoides*, and *B. cereus* have already died after 5-minute exposure to that compound (Fig. 1).

The more intense activity of Steridial P, noted in comparison with pure peracetic acid, may be due to the presence of hydrogen peroxide in the preparation, as the latter also reveals antibacterial activity. In the case of Gram-negative bacteria tested, it has also been demonstrated that peracetic acid kills them totally after a 5-minute exposure (Fig. 1). The choice of *E. coli* and *Ps. aeruginosa* was not accidental, as both spores producing bacteria belong to the group of bacteria, which most often cause nosocomial infections (hospital-acquired infections). Whereas *E. coli* is a bacterium causing relatively insignificant problems, if the infection takes place. *Ps. aeruginosa* is an extremely dangerous bacterium, resistant to numerous antibiotics, causing sepsis in numerous cases, while its ability to become drug-resistant makes fighting it even harder [16, 33].

Glutaralaldehyde is another effective compound used in decontamination [3], in case of using anthrax as biological weapon, it is advised to use 5% glutaralaldehyde for effective disinfection [11].

Staniszewska *et al.* [27] stated that glutaralaldehyde in the concentration of 2% reduces *B. cereus* spores within 15–30 minutes by 4 orders of magnitude, being less effective in the case of *B. subtilis*, and in identical concentration can reduce the number of spores in the course of 30 minute exposure only by 1 log. An increase of temperature from room temperature to 40°C results in increasing the reduction of endospores to 6 orders of magnitude within 30 minutes. On the other hand, 1.5% glutaralaldehyde in the presence of organic impurities in the environment causes killing of *B. subtilis* spores as late as after 8 hours. In the case of *B. cereus* even a 10-hour exposure is not effective [27].
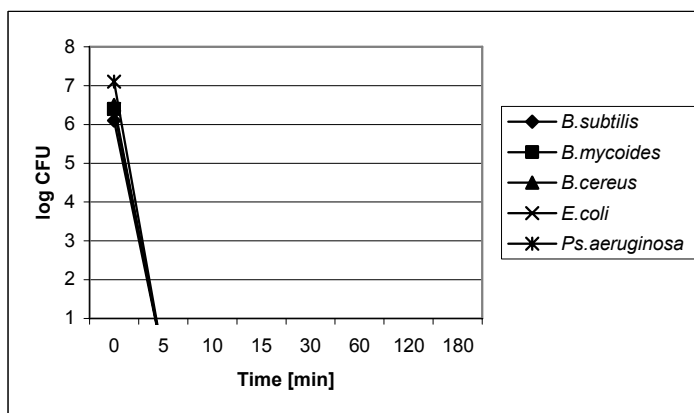

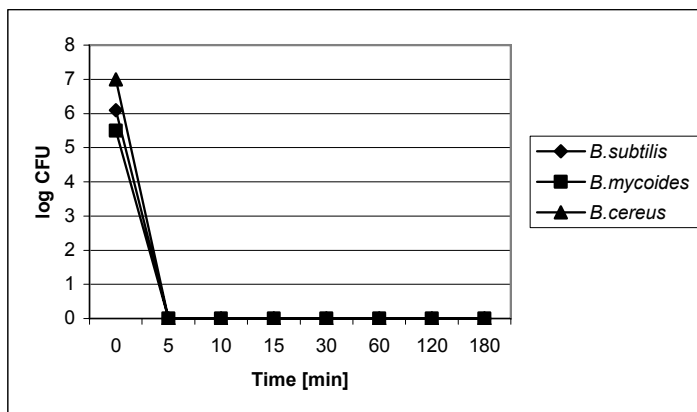Fig. 1. The influence of Steridial P upon the tested strains

Fig. 2. The influence of Lysoformin 3000 upon the tested strains

The results of the study carried out were different, and demonstrated that Lysoformin 3000 with glutaraldehyde as its main active substance reduces totally the spores of all three strains, namely *B. subtilis*, *B. mycoides*, and *B. cereus* already after 5-minute exposure (Fig. 2).

Such major differences in the results obtained are undoubtedly related to the composition of Lysoformin 3000, where, although the main active substance is glutaralaldehyde, there are also other compounds considered disinfectants, such as didecyldimethylammonium chloride, isotridecanol ethoxylated, and glyoxal. Due to the drawbacks and limitations in disinfection, it is believed that multi-component disinfectants are optimal solution, as they combine the active component with components that have washing properties or are emulgators [19].

Also 30% hydrogen peroxide is a strong disinfectant. In the research carried out, the spores of B. subtilis, *B. mycoides*, and *B. cereus* were killed after a mere 5-minute exposure (Fig. 3).

The reduction of hydrogen peroxide concentration from 30% to 5% did not cause reduction of its effectiveness towards the strains examined. The 5% concentration resulted in the same sensitivity of all the three strains examined. The spores of *B. subtilis*, *B. mycoides*, and *B. cereus* were reduced totally after only 5 minutes of exposure (Fig. 4). As in the case of the Bacillus spores, the total reduction of *Escherichia* and *Pseudomonas* bacteria cells occurred after only 5 minutes of exposure (Fig. 4)

Hydrogen peroxide, used for many years for wound disinfection has been rediscovered [8], due to good cleaning properties: in the presence of organic substances a violent decomposition takes place connected with production of free oxygen, which has strong biocidal activity. The scope of its activities includes bacteria, fungi (yeast-like and moulds), mycobacteria, capsid and non-capsid viruses, and in some cases also the spores of *Bacillus subtilis* and *Clostridium difficile* [8]

The results obtained in the study of B. anthracis spores by Mizak were different. The author noted a reduction of spore number by merely one order of magnitude, after 5-minute exposure to 30% hydrogen peroxide, while after 60 minutes another reduction
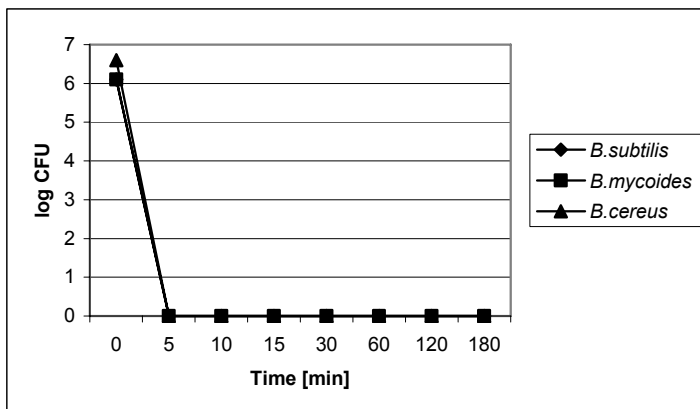
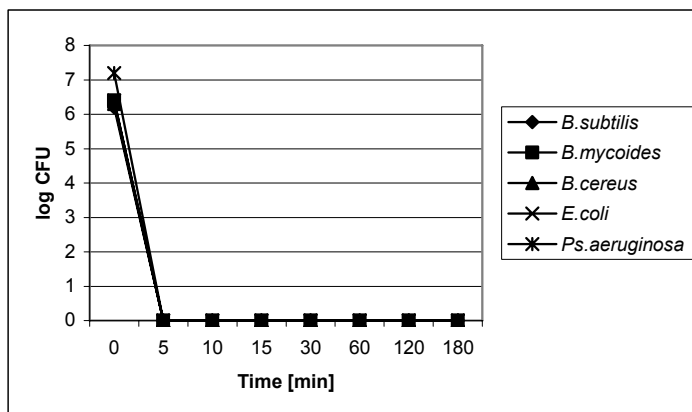Fig. 3. The influence of 30% hydrogene peroxide upon the tested strains

Fig. 4. The influence of 5% hydrogen peroxide upon the tested strains

occurred, also by one order of magnitude. Only a 180-minute exposure eventually killed the spores of *B. anthracis* completely [13].

Similarly, Mizak [13] obtained different values in the study with the application of 4% hydrogen peroxide towards *B. anthracis*, where no sensitivity of the strain to the compound studied was detected. Even a 180-minute exposure did not annihilate the spores, reducing them only by 1 log (from the initial 8 log). Those differences result probably from the exceptional properties of the *B. anthracis* strain, whose spores are especially resistant to external agents [11, 13, 30]

Phenol is a compound, which allows to assess the effectiveness of disinfectants, particularly new preparations, from the point of view of potency in narrow/wide spectrum. They are assessed by means of the so-called phenol index, the value of which is determined by dividing the highest value of dilution of the preparation examined, at which all bacteria are killed within 10 minutes; yet that time cannot be shorter than 5 minutes, by the phenol

dilution, at which the same bactericidal effect occurs. In this way, one can assess how many times the examined preparation is stronger/weaker than phenol [1]. It is believed, however, that phenol and its derivatives are not effective as regards affecting endospores of bacteria [3, 5, 6].

In the performed studies concerning the preparation Rafasept, differentiation has been found in reactions of specific strains. It has been demonstrated that Rafasept, whose main active substance is o-phenyl phenol (ortophenyl phenol), reduces totally the *B. subtilis* spores as quickly as after 5-minute exposure (Fig. 5). On the other hand, the action of Rafasept has not been as effective in relation to the other two strains generating spores. The number of spores, of both *B. mycoides* and *B. cereus* was not reduced, even after 180-minute exposure (Fig. 5). It is of interest, as Rafasept is used for disinfection in hospitals, treatment rooms, outpatient clinics, emergency rescue stations, pharmacies, dental clinics, gynecological examination rooms, beauty parlors, hairdressers, etc.

Such a differentiated sensitivity to o-phenyl phenol is probably species dependent, as sometimes spores of various strains react differently to the same disinfectant in the environment. For example, *B. subtilis* is more resistant than *B. cereus* to disinfectants based on chlorine [27], in turn, *B. cereus* is more resistant to peracetic acid than *B. subtilis* [4].

Another disinfectant that has been tested was the preparation named Promanum N, alcohol-based preparation for surgical and hygienic disinfection of hands, the main ingredient of which is ethanol. The disinfectant proved to be completely ineffective in dealing with spores of all the three strains tested. Even after 180 minutes of exposure, no reduction of spores was noted (Fig. 6).

That result is in compliance with literature, which states that ethanol is one of the two alcohols that are least effective in dealing with bacteria [3], as well as with the producer's recommendations, that suggest the use of the preparation in Activity Area A (Destruction of vegetative forms of bacteria, including micro-bacteria, fungi, and their spores) [producer's leaflet].

Isopropanol is, besides ethanol, among the alcohols most often used in disinfection. Information concerning the effectiveness of this compound vary, however. Różalski [20] states that Isopropanol is the alcohol having the strongest action of all, while the cidal strength of alcohols increases with the number of carbons in chain, whereas Dzierżanowska and Jeliaszewicz [3] state that Isopropanol and ethanol are the least toxic alcohols.

In the study performed, Isopropanol killed vegetative forms of *E. coli* and *Ps. aeruginosa* bacteria after 5 minutes, while it had no influence upon the number of spores of all strains tested, even after 180-minute exposure (Fig. 7) which entails that propanol alcohol is effective towards non-sporing bacteria, while it is completely non toxic for endospores of bacteria of the *Bacillus spp*.

CONCLUSIONS

The following conclusions have been made on the basis of the studies conducted and results obtained:

✓ Within a single genus of spore producing bacteria, sensitivity to the same disinfectant may vary.

✓ Oxidizing compounds (H_2O_2), including those based upon peracetic acid − Steridial P – occurred to be most powerful for fighting bacterial spores.
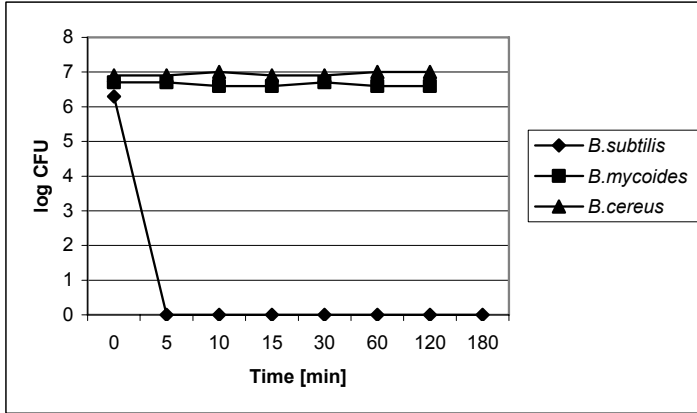
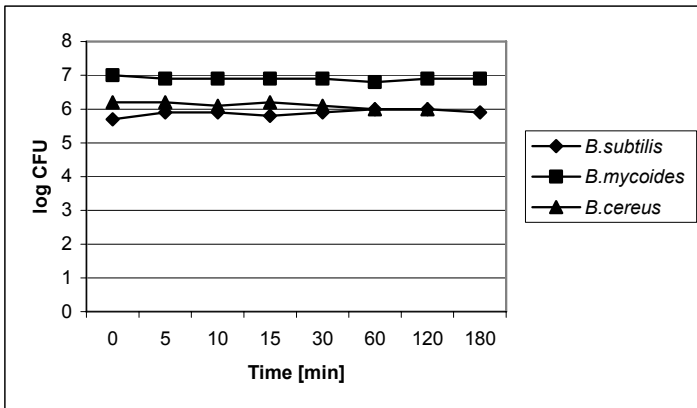Fig. 5. The influence of Rafasept upon the tested strains

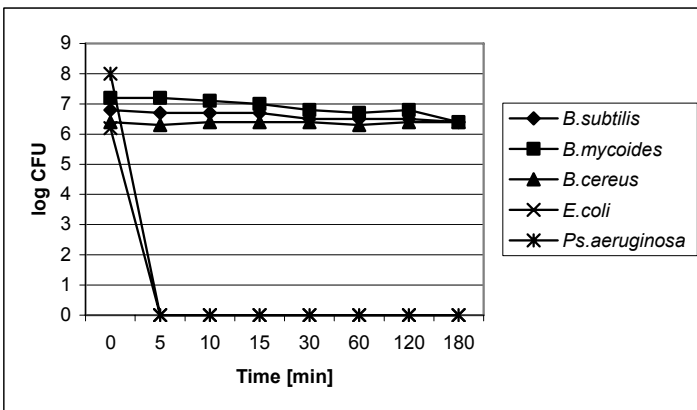Fig. 6. The influence of Promanum N upon the tested strains

Fig. 7. The influence of Isopropanol upon the tested strains

✓ Multi-component preparations are more effective, thanks to coupling various action mechanisms, and are characterized by a wider spectrum of effects.

✓ When applying preparations based on alcohols for disinfection of hands, Isopropanol (98%) and Promanum N, one should expect infections caused by sporing bacteria to occur; that is why the use of multi-component preparations should be advised, as they have wider spectra of effects.

REFERENCES

[1] Bobrowski M.M.: *Podstawy biologii sanitarnej*; Białystok 2002.

[2] Chapman J.S.: *Characterizing bacterial resistance to preservatives and disinfectants*; International Biodeterioratiol and Biodegradation, **41**, 241–245 (1998).

[3] Dzierżanowska D., J. Jeliaszewicz: *Zakażenia szpitalne*; Wydawnictwo Alfa-media press, Bielsko-Biała 1999.

[4] Hilgren J., K.M. Swanson, F. Diez-Gonzales, B. Cords: *Susceptibilities of Bacillus subtilis, Bacillus cereus and avirulent Bacillus anthracis sporesto liquid biocides*; Journal of Food Protection, **72 (2)**, 360–364 (2009).

[5] Kieć-Świerczyńska M.: *Środki odkażające w zakładach służby zdrowia. Rodzaj i częstość stosowania;* Medycyna Pracy, **46 (1)**, 39−46 (1995).

[6] Krzywicka H., A. Bielicka, J. Janowska, E. Jaszczuk, B. Tadeusiak: *Zastosowanie chemicznych środków dezynfekcyjnych w szpitalach*; Państwowy Zakład Wydawnictw Lekarskich, Warszawa 1986.

[7] Kutrowska E.: *Kwas nadoctowy w nowoczesnych procesach dekontaminacji*; Zakażenia, **5 (4)**, 15−19 (2005).

[8] Kutrowska E.: *Nowe rozwiązania w dezynfekcji narzędzi i powierzchni oraz ich praktyczne zastosowanie*; Zakażenia, **3**, 7−13 (2010).

[9] Libudzisz Z., K. Kowal, Z. Żakowska: *Mikrobiologia techniczna*, t. 2; Wydawnictwo Naukowe PWN, Warszawa 2008.

[10] Litwińska B., A. Trzcińska: *Aktywność wirusobójcza preparatów alkoholowych zawartych w środkach przeznaczonych do dezynfekcji rąk (antyseptykach)*; Zakażenia, **3**, 60−67 (2005).

[11] Matras J., M. Bartoszcze, *Bacillus anthracis*; Postępy Mikrobiologii, **41**, **1**, 3−19 (2000).

[12] Meyer B.: *Kwas nadoctowy jako substancja czynna w dezynfekcji*; Aseptyka, **2**, 10–11 (2002).

[13] Mizak L.: *Sporobójcza aktywność nadtlenku wodoru i kwasu nadoctowego wobec przetrwalników Bacillus anthracis*; Medycyna Doświadczalna i Mikrobiologia, **57**, 437−442 (2005).

[14] Murtough S.M., S.J. Hiom, M. Palmer, A.D. Russell: *Biocide rotation in the healthcare setting:is there a case for policy implementation?*, Journal of Hospital Infection, **48**, 1–6 (2001).

[15] Parnowska W.: *Środki odkażające – rola w profilaktyce i zwalczaniu zakażeń szpitalnych*; Farmacja Polska, **54 (15)**, 675−684 (1998).

[16] Parnowska W.: *Znaczenie stosowania i badań skuteczności środków dezynfekcyjnych w profilaktyce zakażeń szpitalnych*; Postępy Nauk Medycznych, **3**, 54−60 (2000).

[17] PN-EN 1040:2000: *Chemiczne środki dezynfekcyjne i antyseptyczne. Podstawowe działanie bakteriobójcze. Metoda badania i wymagania* (faza 1).

[18] PN-EN 14347: 2005: *Chemiczne środki dezynfekcyjne i antyseptyczne – Podstawowe działanie sporobójcze – Metoda badania i wymagania* (faza 1, etap 1).

[19] Rogalewicz R.: *Wieloskładnikowe preparaty dezynfekcyjne – nowe trendy w dziedzinie dezynfekcji instrumentów i powierzchni medycznych w placówkach służby zdrowia*; Pielęgniarka Epidemiologiczna, **4 (43)**, 30–32 (2010).

[20] Różalski A.: *Ćwiczenia z mikrobiologii ogólnej*; Wydawnictwo Uniwersytetu Łódzkiego, Łódź 2003.

[21] Russel A.D.: *Bacterial Spores and Chemical Sporicidal Agents*; Clinical Microbiology Reviews, Apr., 99−119 (1990).

[22] Russel A.D.: *Activity of biocides against mycobacteria*; Journal of Applied Bacteriology, **81**, 87−101 (1996).

[23] Russel A.D., J.R. Furr, J.Y. Maillard: *Microbial susceptibility and resistance of biocides*; ASM News, vol. **63**, no **9**, 481−487 (1997).

[24] Russell A.D.: *Mechanisms of antymicrobial action antyseptic and disinfectants: an increasingly impor-
tant area of investigation*; Journal of Antimicrobial Chemoterapy, **49**, 597−599 (2002).

[25] Russell A.D.: *Bacterial adaptation and resistance to antiseptics, disinfectants and preservatives is not
a new phenomenon*; Journal of Hospital Infection, **57**, 97–104, (2004).

[26] Sagripanti J-L. , Bonifacino A.: *Bacterial spores survive treatment with commercial sterilants and disin-
fectants*; Applied and Enviromental Microbiology, Sept., 4255–4260 (1999).

[27] Staniszewska M., E. Rohm-Rodowald, B. Jakimiak: *Działanie sporobójcze środków dezynfekcyjnych*;
Zakażenia, **6 (5)**, **12**, 14−17 (2006).

[28] Swoboda-Kopeć E., M. Łuczak, B. Łukomska, W.L. Olszewski, S. Jamal, M. Manokaran, E. Stelmach,
E. Zalewska: *Bakteryjne zakażenia skóry i tkanek miękkich w filariozie; Medycyna Doświadczalna
i Mikrobiologia*, **51**, 347−355 (1999).

[29] Walsh S.E., J-Y. Mailard, A.D. Russell: *Ortho-phthaldehyde: a possibility alternative to glutaraldehyde
for high level disinfection*; Journal of Applied Microbiology, **86**, 1039−1046 (1999).

[30] Zaremba M., J. Borowski: *Mikrobiologia lekarska dla studentów medycyny*, PZWL, Warszawa 2001.

[31] Żakowska Z., H. Stobińska: *Mikrobiologia i higiena w przemyśle spożywczym*; Wydawnictwo Naukowe
Politechniki Łódzkiej, Łódź 2000.

[32] www.pis.lodz.pl/pliki/akt20070928.

[33] www.teraz-zdrowie.pl/zakazenia-szpitalne.

AKTYWNOŚĆ WYBRANYCH DEZYNFEKTANTÓW WOBEC PRZETRWALNIKÓW BAKTERII
Z RODZAJU *BACILLUS*

Środki dezynfekujące wykorzystywane są powszechnie w gospodarstwach domowych, w szpitalach, przy pro-
dukcji leków, w przemyśle spożywczym. Przy ciągle narastającej antybiotykooporności mikroorganizmów waż-
ne jest racjonalne stosowanie dezynfektantów w odpowiednich stężeniach, z odpowiednią substancją czynną,
ponieważ nie wszystkie substancje działają w taki sam sposób na różne organizmy. Szczególnie trudnymi do za-
bicia mikroorganizmami są bakterie produkujące spory − formy o odmiennej budowie i odmiennej wrażliwości
na dezynfektanty niż formy wegetatywne. Celem pracy było zbadanie wpływu często stosowanych związków
dezynfekcyjnych na przetrwalniki bakterii z rodzaju *Bacillus: B. cereus, B. mycoides* i *B. subtilis*. W badaniach
nad środkami dezynfekcyjnymi stwierdzono, że najlepszymi dezynfektantami wobec spor są: kwas nadoctowy,
nadtlenek wodoru zarówno w stężeniu 30%, jak i 5% oraz Lysoformin 3000. Najmniej skutecznymi wobec
przetrwalników okazały się Izopropanol i Promanum N. Stwierdzono zróżnicowanie w reakcji poszczególnych
gatunków na preparat Rafasept, ponieważ *B. subtilis* okazał się bardzo wrażliwy na ten związek, natomiast
wobec *B. mycoides* i *B. cereus* Rafasept okazał się nieskuteczny.