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Microbial Sterilization of Talcum Powder Using Gamma Ray (Co-60)

Sterilisasi Mikroba Bubuk Talc Menggunakan Sinar Gamma (Co-60)

Misriyani[1], Bambang Poerwadi[2]

[1]Department of Medical Education, Faculty of Medicine, University of Alkhairaat, Jl. Diponegoro 39, Palu 94221, Indonesia

[2]Department of Chemical Engineering, Faculty of Engineering, Universitas Brawijaya, Jl. Mayjen Haryono 167, Malang 65145, Indonesia

E-mail: misriyani85@gmail.com

ABSTRACT

The damage of talcum powder products is generally caused by microbial growth. Some industries overcome this problem using gamma ray irradiation preservation technique (Cobalt-60). This study identifies the number of microbes remaining in talcum powder and the structure of irradiated bacterial cells. In the preliminary study, the irradiation process was conducted by providing an irradiated dose of talcum powder consisting of 4 levels, namely 0 kGy, 5 kGy, 7 kGy, and 9 kGy at a constant speed of 7 m per minute and a cycle time of 5 minutes using a gamma ray irradiator (Co-60), and continued with microbiological tests on the remaining microbes in the irradiated talcum powder. The test consisted of counting the number of bacteria using the plate count method and identification of the dominant bacterial cell structure using the Gram stain method and microscopic observation. The results showed that the number of bacteria in talcum powder decrease with the increasing dose of irradiation. The total count *P. aeruginosa* before and after irradiation count were 11.0 x 10^4 and 2.8 x 10^4 colony forming units (CFU) per gram with control sample (0 kGy) respectively. Identification of the dominant bacterial cell structure in the irradiated talcum powder shows the characteristics of the *P. aeruginosa P. aeruginosa*bacteria with a round, smooth, white colony, giving greenish pigment to the medium, and showing Gram-negative bacterial cells, with a stem structure, sometimes coupled and irregularly branched and red.

Keywords: Talcum powder, microbe, gamma ray irradiation

ABSTRAK

Kerusakan produk bedak talk umumnya disebabkan oleh pertumbuhan mikroba. Beberapa industri mengatasi masalah ini dengan menggunakan teknik pengawetan iradiasi sinar gamma (Cobalt-60). Penelitian ini mengidentifikasi jumlah mikroba yang tersisa dalam bedak talk dan struktur sel bakteri yang diiradiasi. Pada studi pendahuluan, proses iradiasi dilakukan dengan memberikan dosis iradiasi bedak talk yang terdiri dari 4 taraf yaitu 0 kGy, 5 kGy, 7 kGy, dan 9 kGy dengan kecepatan konstan 7 m per menit dengan *cycle time* masing-masing 5 menit. menggunakan sinar gamma irradiator (Co-60), dan dilanjutkan dengan uji mikrobiologi terhadap sisa mikroba dalam bedak talk yang diradiasi. Pengujian terdiri dari penghitungan jumlah bakteri dengan metode *plate count* dan identifikasi struktur sel bakteri dominan menggunakan metode pewarnaan Gram dan pengamatan mikroskopis. Hasil penelitian menunjukkan bahwa jumlah bakteri pada bedak talk menurun seiring dengan meningkatnya dosis iradiasi. Jumlah total *P. aeruginosa* sebelum dan sesudah iradiasi masing-masing adalah 11,0 x 10^4 dan 2,8 x 10^4 CFU per dengan sampel kontrol 0 kGy. Identifikasi struktur sel bakteri yang dominan pada bedak talk yang diiradiasi menunjukkan ciri-ciri bakteri *P. aeruginosa* dengan koloni bulat, licin, putih, memberikan pigmen kehijauan pada medium, dan menunjukkan sel bakteri Gram negatif, dengan struktur batang, kadang-kadang bergandengan dan bercabang tidak beraturan dan berwarna merah.

Kata kunci: Bedak talk, mikroba, iradiasi sinar gamma

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INTRODUCTION

According to Weinkauf (2013), some products made from talcum powder consist of baby powder, health powder, cosmetics, deodorant, colored pencils, textiles, soap, paint, and paper. Due to its anhydrous nature, talc used as an ingredient in the pharmaceutical industry in the manufacture of drugs [1] .

Talcum powder on the market is rarely found as a raw material, but as a product that can be directly used. Cosmetics is one of the commodities that uses talcum powder as the main component, and is widely used for body care and make-up. For this reason, it is necessary to carry out stages so that the cosmetics produced are always safe, quality and useful. The safety and quality of cosmetics depend on raw materials, packaging materials, infrastructure, production processes, quality control, equipment used and labor involved in production [2] [3]. One of the causes of cosmetic damage is the presence of microbial contamination, both fungi and bacteria, originating from the environment such as water, raw materials used, and production rooms [4]. In the market, it may be affected by inadequate storage and packaging conditions for cosmetics [5].

Several techniques are carried out to protect products from microbial contamination that can harm and endanger consumers, such as using sterile raw materials, aseptic manufacturing, and the declaration of the Decree of the Director General of Drug and Food Control of the Ministry of Health of the Republic of Indonesia No: HK .00.06.4.02894 Regarding Requirements Microbial Contamination in Cosmetics [6].

A technique for cleaning tools or materials from all kinds of living things, especially microbes, is called sterilization. Sterilization is carried out in various ways, one of the sterilization techniques used in the Indonesian industry is to use Cobalt-60 gamma ray irradiation as ionizing radiation [7]. Microbes that survive after passing through radiation, they remain in a damaged state and are very sensitive to growth-inhibiting states during storage (e.g., low temperatures) and die easily due to heating. Pathogenic microbes that survive radiation can cause public health problems, not because the irradiation process has changed them, but because the bacteria are still alive [8].

To determine the remaining microbial population on a material that has been sterilized. The number of microbes was calculated using the plate count method, as well as the Gram staining method and observation using a microscope to determine the structure of bacterial cells. In this case, we will discuss in more detail about the plate counting method, the Gram stain method and the observation using a microscope on samples of irradiated talc powder.

METHODOLOGY

Equipment/ Tool/ Material

The materials used in this study were talcum powder, agar nutrient media, sterile distilled water, distilled water, 95% alcohol, crystal violet dye, iodine, and safranin dye. The tools used in this research include the main facilities and supporting facilities for the irradiation system which was carried out at PT. Rel-ion Bekasi using a gamma ray irradiator (Co-60), balance, autoclave, incubator, petri dish, mechanical counter, 500 mL beaker, test tube, heater, 10 mL measuring pipette, 1 mL measuring pipette, bunsen, cotton ball, oven spatula, inoculation loop, slide, dropper, and microscope.

Experiment

Microbial identification of talcum powder as a result of gamma ray radiation using the plate count and Gram staining method is as follows:

Irradiation process

Four samples of raw talcum powder were prepared with a mass of 200 g. Each sample was wrapped in plastic, sealed to airtight and marked with the irradiation dose with varying doses of 5 kGy, 7 kGy, 9 kGy and samples without irradiation as a control. The dosimeter was attached to the sample and put in a tote in a carrier connected to a conveyor system for sterilization at a constant speed of 7 m per minute and a cycle time of 5 minutes. Figure 1 shows the conveyor and tote in the irradiation chamber. Sterile samples are then carried out for microbial identification which was carried out at the Biochemistry Laboratory of Science Faculty, Universitas Brawijaya. Microbial identification is carried out under sterile conditions. Experimental materials and equipment were sterilized in an autoclave and dry oven.

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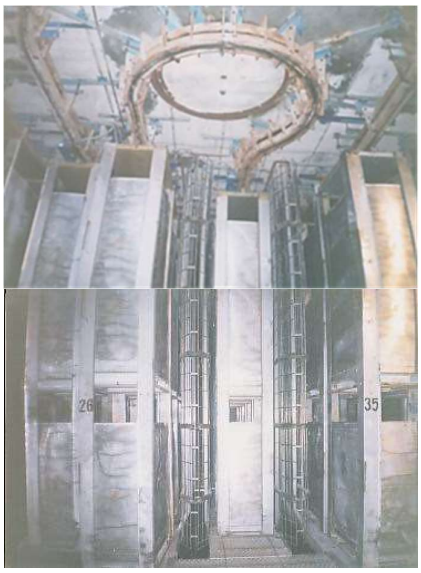

Figure 1. Conveyor and tote grooves in irradiation chamber

Preparation of medium and preparation of dilution series

Medium preparation

The media is made by mixing 1.2 g of beef extract, 0.6 g of peptone, and 15 g of agar. Then dissolved with 120 mL of distilled water, and heated to boiling while stirring until completely dissolved. After that, 10 mL of the medium was directly pipetted and put into 12 test tubes. The medium was covered with cotton and wrapped for sterilization.

Preparation of dilution series

5 g of talcum powder was aseptically put into 45 mL of sterile 0.9% peptone solution and homogenized using a vortex. This homogeneous sample is called the 10^{-1} dilution series. Then 1 mL of the sample was put into a test tube containing 9 mL of a sterile 0.9% peptone solution and homogenized with a vortex. This sample is called the 10^{-2} dilution series. The same treatment was continued to obtain samples of the 10^{-2}, 10^{-3}, and 10^{-4} dilution series.

Bacterial isolation and incubation

A petri dish containing 0.1 mL of sample with serial dilutions of 10^{-2}, 10^{-3}, and 10^{-4}, was added 10 mL of sterile medium at a temperature of 50°C aseptically and evenly. The solid medium was then incubated with the cup upside down in an incubator at room temperature for 48 hours.

Counting the number of colonies

After incubation, the number of colonies on the petridish from each dilution was counted directly and accurately. Colony counts are based on standard plate counts. If the number of colonies per plate in petri dish replications or successive dilutions is averaged, then only those that meet the requirements are averaged. If the number of plates from two levels of dilution produces colonies with an amount between 30 and 300, then the calculation is based on the ratio between the highest and lowest diluent, if the ratio is less than 2 then the results are averaged, while if it is greater than or equal to 2 then the same dilution is used. If in all dilutions less than 30 colonies were produced per petri dish, then the number of colonies in the lowest dilution was counted. Calculation of the number of bacterial cells per gram of talcum powder using the formula:

$$\text{Colonies } (mL^{-1} \text{ or } g^{-1}) = \text{colonies/plate x } \frac{1}{FP} \quad (1)$$

Results are reported as colony-forming unit per gram (cfu per g).

Gram stain and microscope observation

Bacterial samples used in Gram staining were made by aseptic transfer of the most dominant living colonies. This procedure was carried out using a loop inoculation into the medium and incubated for 24 hours to obtain pure cultures. Furthermore, the bacterial smear was carried out by dripping 1-2 loops of sterile distilled water on a sterile slide followed by transferring a small amount of the culture over the water droplets evenly. The smear was air-dried and fixed on a Bunsen flame.

After completion of heat fixation; 1) flood the bacterial smear with crystal purple primary dye for 1 minute, remove excess purple crystals and rinse with distilled water, 2) soak the smear with iodine for 2 minutes, then rinse with distilled water, 3) wash the smear by dripping with 95% alcohol bleach for 30 seconds or until the crystal purple dye is no longer visible from the slide, rinse with distilled water, 4) flood the smear with safranin counter-dye for 30 seconds, remove excess safranin and rinse with aquadest. Air dry, and observe using a 400x magnification microscope. Note the shape and color of the bacterial cell.

RESULT AND DISCUSSION

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Effect of irradiation dose on bacterial cell count

A dose of irradiation will kill the microbial population present in a substance. The larger the bacterial population that is before irradiation, the more bacteria will remain after irradiation. According to Correa et al. 2019 irradiation inactivates bacteria by killing or inhibiting metabolic activity [9] The bacterial population decreases or does not exist at all with the increasing dose of irradiation given to the material [8][10][11]. The results data and the calculation of the number of bacterial cells per gram of talcum powder sample at various irradiation doses using the plate count method are shown in Figure 2.

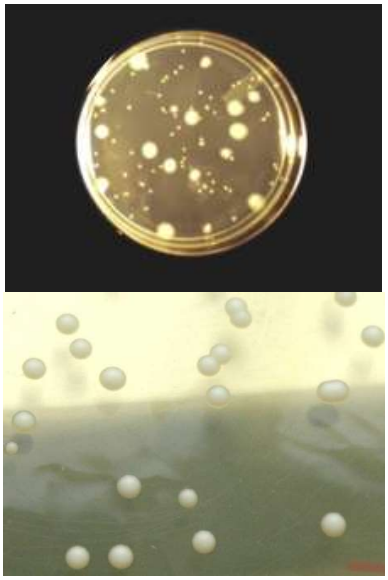

Figure 2. The dominant form of bacterial colonies of *P. aeruginosa*

Table 1. Results of counting bacteria per gram of talcum powder

Irradiation dose	colony-forming unit per gram (cfu per g)	SD
0 kGy	110,000	2.8
5 kGy	73,000	4.2
7 kGy	42,000	1.4
9 kGy	28,000	2.8

The basis for calculating the number of bacterial cells per gram or per mL of sample using the plate count method is done by making a series of dilutions of bacterial samples in multiples of 10, then from each dilution 1 mL is taken and grown on agar medium in a petridish [12]. The function of dilution is to reduce the number of microorganisms, so that at least one cup contains the appropriate number of colonies. In addition, it facilitates the calculation of the number of colonies from samples that may contain more than 300 microbial cells.

Data on the number of bacterial cells per gram of talcum powder showed that at the highest irradiation dose of 9 kGy, microbes that survived irradiation were still found, so it was possible for microbes to die at higher irradiation doses. To determine the value of the irradiation dose to reduce biological effects, especially microorganisms to a lethal dose, an equation of the regression line was performed on the graph of the relationship between the irradiation dose and the number of bacterial cells per gram of sample as follows.

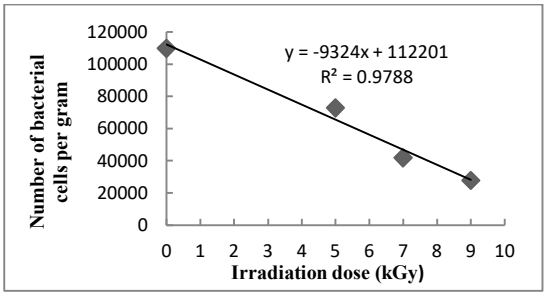

Figure 3. Effect of irradiation dose on the number of bacterial cells per gram of talcum powder

The graph in Figure 3 shows that the number of microbes in the talcum powder sample will decrease with increasing the dose of irradiation exposed. This is due to the very strong ability of irradiation to kill cells, so that every bacterium, germ and microbe will die by exposure to strong irradiation [13].

To find out the regression line is feasible to use, it can be seen in the value of the coefficient of determination R^2. If R^2 (can also be displayed in %) is close to 1 (or 100%), then the regression model is quite good, whereas if R^2 is close to 0 (or 0%), then the regression model is not good enough to be used [14]. From the graph, the R^2 value is close to 1 (or 100%) which is 0.9788. From the graph, the regression line equation y= -9324x + 112201 is obtained, which can be used to calculate the lethal dose to kill microorganisms as a whole.

Considering that talcum powder is not a type of food ingredient, the amount of irradiation dose has a low effect on product quality, in contrast to food ingredients that are damaged in quality if the irradiation dose exceeds the maximum dose, so it is not accepted by consumers. Determination of the right irradiation dose is necessary for the safety of

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talc products by considering product quality, cost, processing time, product density, cycle time and product configuration on the tote.

The size of the irradiation dose received by a sample is influenced by several factors, among others: cycle time. Cycle time is defined as the time of transfer of radiation from one carrier position to another carrier position in one rotation. There are 45 carrier positions in the process. Talcum powder samples used a cycle time of each irradiation dose of 5 minutes, so that the time needed for one cycle was approximately 3 hours 45 minutes. In addition, the amount of irradiation dose received by the sample is also influenced by the determination of the dose map at 27 positions, namely 9 upper positions, 9 middle positions, and 9 lower positions of the product configuration in the tote. Because the position of the iradition source is on both sides of the tote, the largest energy absorption is at the location closest to the source and the smallest energy absorption is at the center of the totProducts based on talc powder are marketed in sterile form, this is in accordance with the decision of the Director General of Drug and Food Control regarding the requirements for microbial contamination in cosmetic products to be negative or free from microbes [15].

From the experimental results, it was found that the microbes that grew on the medium and the large standard deviation values were caused by factors during processing, starting from sterilization of tools and materials, manufacture of medium and dilution series, isolation of bacteria and incubation, until the calculation of colonies on the dish was less precise. Sterilization of tools and materials that are not optimall, produce unwanted microbes. Different medium and incubation conditions may produce different values. Non-aseptic bacterial isolation treatment causes the growth of unwanted microbes into petri dishes, as well as inaccurate calculations and not the actual number of cells. Because it is possible for several adjacent cells to form a colony or the presence of colonies in very small sizes cannot be counted, thereby increasing the standard deviation value [16].

Identification of the dominant bacterial cell structure from irradiation by colony observation, Gram staining and microscopy

Bacteria are unicellular microbes, do not have chlorophyll, and reproduce asexually through cell division. The size of the bacteria in microns is difficult to observe with an ordinary microscope. Bacterial cell walls are divided into two types which are the basis for classification through Gram staining.

Observations of colonies growing in petri dishes were obtained after incubation for 2 x 24 hours at room temperature of 26 ± 10^0 C using nutrient agar as a medium. The conditions of time, temperature and media are very suitable for growing bacteria. From the test results, samples with a dilution series of 10^{-1} almost all of the plates contained more than 50% colonies with round and white colonies, the number of colonies was estimated to be more than 300 colonies and it was very difficult to count, so they were not included in the calculation.

The number of colonies can easily be observed and counted after several dilutions. Figure 2 is one of the dishes with the number of colonies that meet the requirements between 30-300. The results showed that the colonies were round in shape, smooth convex edges and white in color, and gave a greenish pigment to the medium. the highest dilution of 10^{-4} causes no live bacteria so that the colonies do not grow on petri dishes, besides that it can also occur in samples with the smallest dilution of 10^{-1}, so it is necessary to make additional dilution series so that the colonies are clearly visible and easy to count.

In the Gram stain test, the results showed that the bacteria were stained with crystal violet dye, but faded when treated with alcohol and stained with safranin to give a pink color. Observation using a microscope with a magnification of 400x, bacteria appear rod-shaped, reddish, sometimes coupled and branched irregularly. The results of these observations showed the type of Gram-negative bacteria, rod-shaped, red in color according to the structure of *P. aeruginosa*. *P. aeruginosa* is a type of bacteria that has advantages compared to other microorganisms. In addition to the extraordinary capacity to form a disease, the bacterium *P. aeruginosa* has the ability to be resistant to most antibiotics, can produce a layer of mud as a protection against germ-killing substances, has the ability to survive in conditions that few other organisms can tolerate and has the ability to survive, to be able to adapt and grow in a fertile environment as well as in an environment with limited nutrients [17] [18] [19] [20].

Based on the results of colony observations, Gram staining and microscopic observations,

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obtained characteristics that match *P. aeruginosa* as a Gram-negative bacterium with a round colony shape, smooth convex edges and white color, and gives a greenish pigment to the medium. So it can be concluded that the dominant type of bacteria contained in the talcum powder sample is *P. aeruginosa*.

CONCLUSION

The number of microbes in the talcum powder sample will decrease with the increasing dose of irradiation given. The calculation results show that, in the sample without the irradiation dose there were 110,000 bacterial cells per gram, the sample with the 5 kGy irradiation dose contained 73,000 bacteria cells per gram, the sample with the 7 kGy irradiation dose contained 42,000 bacterial cells per gram and the sample with the irradiation dose 9 kGy contains 28,000 bacterial cells per gram. Identification of the dominant bacterial cell structure contained in talcum powder by observing the colony form, microscopy and Gram staining, shows the characteristics of the *P. aeruginosa* bacteria.

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REFERENCES

[1] J. G. Weinkauf *et al.*, "Long-term Outcome of Lung Transplantation in Previous Intravenous Drug Users with Talc Lung Granulomatosis," *Transplant. Proc.*, vol. 45, no. 6, pp. 2375–2377, Aug. 2013, doi: 10.1016/j.transproceed.2012.11.004.

[2] "Keputusan Menteri Kesehatan Republik Indonesia Nomor : 965/Menkes/Sk/Xi/1992 Tentang Cara Produksi Kosmetika yang Baik Keputusan-menteri-kesehatan-republik-indonesia-nomor-965-menkes-sk-xi-1992-tentang.html (accessed Oct. 25, 2021).

[3] B. Sawicka, T. Hameed, A. Noaema, and A. Kiełtyka-Dadasiewicz, "Safety of Plant Cosmetic Raw Materials," *Int. J. Res. Methodol. Soc. Sci.*, vol. 2, pp. 6–22, 2016.

[4] N. Halla *et al.*, "Cosmetics Preservation: A Review on Present Strategies," *Mol. J. Synth. Chem. Nat. Prod. Chem.*, vol. 23, no. 7, p. 1571, 2018.

[5] L. Dadashi and R. Dehghanzadeh, "*Investigating Incidence of Bacterial and Fungal Contamination in Shared Cosmetic Kits Available in the Women Beauty Salons*," *Health Promot. Perspect.*, vol. 6, no. 3, pp. 159–163, 2016.

[6] "Keputusan Direktur Jenderal Pengawasan Obat Dan Makanan Departemen Kesehatan Republik Indonesia Nomor: Hk.00.06.4.02894 Tentang Persyaratan Cemaran Mikroba pada Kosmetika Direktur Jenderal Pengawasan Obat Dan Makanan." https://www.regulasip.id/book/9195/read (accessed Oct. 25, 2021).

[7] K. Aquino, "Sterilization by Gamma Irradiation," 2012.

[8] L. McKeen, "Introduction to Food Irradiation and Medical Sterilization," *Eff. Steriliz. Plast. Elastomers*, pp. 1–40, 2012.

[9] W. Correa *et al.*, "Inactivation of Bacteria by γ-Irradiation to Investigate the Interaction with Antimicrobial Peptides," *Biophys. J.*, vol. 117, no. 10, pp. 1805–1819, 2019.

[10] "Food Preservation by Irradiation," in *Food Microbiology: Principles into Practice*, John Wiley & Sons, Ltd, pp. 106–126. 2016

[11] W. A. Rutala, "Guideline for Disinfection and Sterilization in Healthcare Facilities," p. 163, 2008.

[12] P. Thomas *et al.*, "Optimization of Single Plate-Serial Dilution Spotting (SP-SDS) with Sample Anchoring as an Assured Method for Bacterial and Yeast cfu Enumeration and Single Colony Isolation from Diverse Samples," *Biotechnol. Rep.*, vol. 8, pp. 45–55, 2015.

[13] A. Seltsam and T. H. Müller, "UVC Irradiation for Pathogen Reduction of Platelet Concentrates and Plasma," *Transfus. Med. Hemotherapy*, vol. 38, no. 1, pp. 43–54, 2011.

[14] M. S. Paolella, *Linear Models and Time-Series Analysis: Regression, ANOVA, ARMA and GARCH*. John Wiley & Sons, 2018.

[15] "WHO Expert Committee on Specifications for Pharmaceutical Preparations," *Rev. Inst. Med. Trop. São Paulo*, vol. 50, no. 3, pp. 144–144, 2008.

10.17146/jair.2022.18.1.6605
p ISSN 1907-0322
e ISSN 2527-6433

[16] K. E. R. Davis, P. Sangwan, and P. H. Janssen, "Acidobacteria, Rubrobacteridae and Chloroflexi are Abundant Among Very Slow-Growing and Mini Colony Forming Soil Bacteria," *Environ. Microbiol.*, vol. 13, no. 3, pp. 798–805, 2011.

[17] J. Botelho, F. Grosso, and L. Peixe, "Antibiotic Resistance in Pseudomonas aeruginosa – Mechanisms, Epidemiology and Evolution," *Drug Resist. Updat.*, vol. 44, p. 100640, 2019.

[18] P. Pachori, R. Gothalwal, and P. Gandhi, "Emergence of Antibiotic Resistance Pseudomonas Aeruginosa in Intensive Care Unit; A Critical Review," *Genes Dis.*, vol. 6, no. 2, pp. 109–119, 2019.

[19] L. Wang, C. Hu, and L. Shao, "The Antimicrobial Activity of Nanoparticles: Present Situation and Prospects for The Future," *Int. J. Nanomedicine*, vol. 12 pp. 1227–1249, 2017.

[20] G. Novik, V. Savich, and E. Kiseleva, *An Insight Into Beneficial Pseudomonas bacteria*. IntechOpen, 2015.