The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2021

Volume 16, Pages 231-239

IConTES 2021: International Conference on Technology, Engineering and Science

Inhibition of the Growth of *Staphylococcus Aureus* by Using Some Antibiotics Produced from *Bacillus Subtilis*

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Abstract: *Bacillus subtilis* can produce many substances that are considered as a means of defense in addition to being tested and approved as antibiotics. The goal of this study was to see if *Bacillus subtilis* crude bacteriocin had any antibacterial effects on *Staphylococcus aureus* .The peptidic bacteriocin is considered of importance in the industrial and medical fields as an anticancer.We got our bacteria from the Bacterial Bank at the College of Science and the Department of Biology. Identification of the isolates was confirmed by microscopically and biochemical tests which include catalase, oxidase, IMViC, blood hemolysis, starch hydrolysis for *Bacillus*, and growth on Mannitol Salt Agar (MSA), Voges-Jenson agar(V.J), and Coagulase test for *Staphylococcus*. Based on the capacity of bacteriocin to diffuse in agar, the reverse technique, which eliminated the contact between the producers and sensitive strains, was used to detect bacteriocin antibacterial action. Results demonstrate the antibacterial effect of Bacillus *subtilis* bacteriocin by the inhibition of *Staphylococcus aureus* as compared to other indicator isolates.

Keywords: *Bacillus.subtilis*, *Staphylococcus.aureus*, Bacteriocin

Introduction

We had the good fortune of being alive during the Golden Age of Antibiotics, which lasted from the mid-1940s until recently. Antibiotics have been used to treat all major bacterial and fungal infections during this period. Surgery has improved dramatically during this time period, which has transformed medicine and saved countless lives. It's a shame that the golden era of antibiotics is coming to an end because pathogens are becoming increasingly resistant to them. Penicillin resistance was immediately noticed and has only gotten worse in the last half-century. Antibiotic resistance is a particular concern when dealing with MRSA. (Methicillin-Resistant *S. aureus*), which is endemic in most hospitals Worldwide (Boyce, et. al., 2005). It's been known since the 1880s when *S. aureus* was first discovered that it's a potentially pathogenic Gram-positive bacterium, capable of inflicting serious infections like postoperative wounds and minor skin infections. Currently, it's the second most common cause of bloodstream and lower respiratory tract infections. It took two years after the introduction of penicillin for medical use before penicillin-resistant *S. aureus* began to appear in hospitals. Penicillin-resistant strains of *Staphylococcus aureus* appeared in the general population only a few years later. (Croft, et.al., 2007; Deurenberg, 2008).

Penicillin-resistant strains of *Staphylococcus aureus* have been extensively studied in Iraq. To put this into context, (Kareem, *et al*., 2015) reported that Medical City in Baghdad provided 74 isolates. MRSA was found in 61 of the isolates. There was also a study by (Al-Dahbi,2013) that found 106 *S. aureus* isolates were found to be completely resistant to PenicillinG (100 percent) and highly resistant to Cefoxitin in nasal swabs from health care workers and patients at Al-Kadhamia Teaching Hospital in Baghdad and Al-Numan Hospital. (Alternative to Methicillin 94.3%). Antibiotic resistance, on the other hand, will remain a major issue in future medicine. The simplest solution to this problem is to create new antibiotics that target pathogens that haven't yet developed

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- Selection and peer-review under responsibility of the Organizing Committee of the Conference

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resistance to existing ones (Mulvey, 2009). After demonstrating significant activity against *S. aureus*, Bacteriocin may be a potential antibiotic for this pathogen (Mulvey, 2009). Antimicrobial peptides, also known as bacteriocins, are one of the most well-studied microbial defense systems, and they can be used to explain microorganism evolution and ecology. Bacteriocins may also serve as a natural alternative to antibiotics in the treatment of bacterial infections and as natural antimicrobials in food preservation (Cleveland, et. al., 2001).

They can inhibit the growth of bacteria of the same species (narrow spectrum) or other genera (broad spectrum), and their range of activity often depends on the mechanisms of action of each Bacteriocin. Bacteriocins are typical alternatives to antibiotics. Their short chains of about 20-60 amino acid residues are usually heat-stable, but longer chains can also be found (Snyder, 2014). As shown in the BACTIBASE dataset, the majority of bacteriocins are derived from Gram-positive bacteria. Because of their broad spectrum antibiofilm activity, Bacteriocins are generally considered to be safe and stable. Bacteriocins from Gram-negative bacteria have been described, but there are none from the Archaea domain. Gram-negative bacteria are rare (Hammami, et. al., 2013).

General Mode of Action

Bacteriocin act as a target by forming membrane pores that disturb the energy potential of sensitive cells. Different bacteria produce different bacteriocins which have their mode of action.

The mode of action of nisin is best studied. These bacteriocins associate electrostatically with phospholipids, which causes the interaction of bacteriocins' hydrophobic residues with the cytoplasmic membrane of target cell (Nicolle & Prunet.,1964). Electrostatic interaction is caused by Lysine (cationic amino acid). Ionic channels are created as a result of the hydrophobic nisin part interacting with the membrane. High transmembrane potentials, anionic lipids, and the absence of cationic lipids favor this theory (O′Sullivan. et. al.,2002). Because divalent cations neutralize the phospholipids' negative charges, the membrane's fluidity is reduced. Pores produced by nisin create passive efflux of K^+ and Mg^{2+}, amino acids, Cell death occurs as a result of ATP and proton-motive-force dissipation. YGNGV, found in the N-terminal region of Class I bacteriocins, makes them highly specific against Listeria monocytogenes (Schwable. et. al.,2007). The current mechanism to explain mode of action antibacterial binding to the target membrane is electrostatic and is caused by a putative membrane-bound receptor molecule, although the need for this particular receptor is still debatable. The YGNGV anti-listerial motif found in these peptides is recognized by an unknown receptor (Cintas, et. al., 1995).

Pathogenic and spoilage microorganisms can be controlled biologically by using antibacterial peptide-producing *Bacillus* species, which is critical for food preservation. Lactic acid bacteria (LAB) found in food and other sources have been the subject of extensive research. Recently, For inhibitory substances, the Bacillus species have also attracted interest because of the large number of antibiotics in the form of peptides they produce, which represent a wide range of chemical structures. Additionally, similar to LAB, some *Bacillus* spp., such as *B. subtilis, B. licheniformis,* and *B. coagulans* are'GRAS' in the food industry and Agriculture. Endospore-forming Gram-positive bacteria make up the family Bacillaceae, which has rod-shaped members. There are two major types of spore-forming bacteria in this family: Clostridium is a genus of anaerobic, spore-forming bacteria.

Bacillus spore-forming bacteria can be aerobic or facultative anaerobic. Bacillus spore bearers are common names for members of the genus Bacillus. They are all around us, in the soil, in the air, and the water. As well as being common pathogens, these bacteria are frequently found to be contaminants in bacterial culture media. It is well-known that *Bacillus* spp. can be used safely in the food industry (Abriouel, et. al., 2011). The US FDA has designated some of the most important Bacillus species, like *B. subtilis* and *B. lichenifornis*, as "generally recognized as safe for sale. (Chopra, et. al., 2015).

Gram-positive Bacillus is widely distributed and has a rod-shaped morphology, making it an endospore-forming bacterium [O'Sullivan,2002]. The bacteria Bacillus can be found in soil and clays as well as rocks, dust, and aquatic environments as well as vegetation, food, and the gastrointestinal tracts of various insects and animals due to endospore formation in adverse conditions. (Nicholson. 2002).

Some Bacillus species have been found to be rich sources of bacteriocins, lipopeptides, and other inhibitory substances that are similar to those found in Bacillus spp. bacteria produce and secrete antimicrobial peptides known as bacteriocins to protect themselves from the growth of other bacterial species that are closely related (Cotter, et. al., 2013). Most bacteriocins work by blocking pore formation on the cell surface or interfering with

cell wall synthesis to slow bacteria's growth. As reported by the (International Nosocomial Infection Control Consortium in 2010), both *Staphylococcus spp*. gram-positive bacteria and *Escherichia coli* gram-negative bacteria can develop resistance to antibiotics (Hashemizadeh , 2011).

A common source of hospital-acquired infection is Gram-positive bacteria. Nosocomial bacteremia has been linked to the bacteria *Staphylococcus epidermidis*. Because of drug resistance, allergic reactions, and other side effects, researchers are now looking into new drugs to combat nosocomial infections in hospitals (Agarwal, et. al., 2016). A class of molecules known as antimicrobial peptides has shown promise as novel therapeutics due to their potency and broad-spectrum. However, a possible role as an alternative antimicrobial agent for infection is being explored for bacterial bacteriocins, which are used in the food industry (Cotter, 2013).

Bacilli are also of major importance in the fermentation industry since they elaborate on a variety of useful enzymes and antibiotics. As a consequence, in this regard, industrial-scale bacilli cultivation techniques have come a long way. Commercial exploitation is particularly interested in the ability of some bacilli to excrete large amounts of specific proteins into extracellular space. It's unfortunate that so little is known about the molecular basis of bacterial protein excretion and whether or not excretion and synthesis are coupled. This, as well as the intriguing issues surrounding growth stage-related regulation, will most likely be the subject of research shortly. To a certain extent, this optimism stems from the discovery of molecular cloning in *Bacillus subtilis* **(**David, 1982).

Bacteriocin production has been detected using a variety of methods. Gratia and Fredricq are the main sources of inspiration for all of the standard techniques. (Agarwal. *et al*.,2016). Bacteriocins can diffuse into solid or semisolid culture media, which are then inoculated with a suitable indicator strain. These techniques take advantage of this fact. Before moving on to the next step, the bacteriocin-producing strain must be removed or killed with chloroform vapors to ensure the agar surface is sterile for inoculating the indicator strain. If the bacteria are still alive and growing on the agar surface, the inhibition areas of the indicator strain may be completely or partially hidden. Plastic Petri dishes cannot be used after the producer strain of bacteria has been killed with chloroform because the vapors attack the plastic. Residual chloroform in the culture medium insufficiently ventilated after exposure to vapors can give erroneous results. (Al-Danbi. *et al*.,2013).

Nicolle and Prunet's approach are described here. not require killing or removing the producer strain, inoculated with a straight wire and incubated for 2 days under the surface of a truncated Agar Slant. The indicator strain is inoculated onto the slant's surface on the third day. the presence or absence of growth of the indicator strain after incubation can be used to record the results. Since each producer or indicator strain requires a separate test tube, this method is impractical for screening. It's based on the fact that bacteriocin diffusion (like that of any other antibiotic) is tridimensional, which is how the new method works. As a result, the substance can be identified on the agar's reverse side. This site will be used as the indicator strain's culture surface. Here's how to do it: Using the standard procedure, the producer strain is inoculated onto nutrient agar's surface (spot inoculation, single colonies, or strip). A sterile spatula is used to remove the agar from the Petri dish's edges after it has been properly incubated. After that, the plate is inverted and the petri dish is thumped hard on the bench to release the agar disc into the top. A new lid places the sterile surface (previously located at bottom of the dish) higher than before, making it possible to inoculate with the indicator strain. Zones of inhibition can clearly be seen after incubation (Nicolle, 1964).

Material and Method

Reagents:

1. Tetramethyl – p- phenylenediamine (DMPD)
2. 3% H_2O_2
3. Plasma

Stains:

1. Gram Stain
2. Malachite green

Media:

1. Nutrient broth
2. Nutrient Agar
3. blood Agar
4. Mannitol salt agar
5. Christensen's urea Agar
6. Voges – Jenson (VJ)

1. Bacterial Isolates:
Two Bacterial Isolates were obtained from the Bacterial bank/college of science / Dept. of Biology. One Isolates of *Bacillus subtilis* and staphylococcus aureus.
2. Identification of bacterial isolates *Bacillus subtilis:* by using the following test:
Gram and spore stain, catalase, oxidase, urease, starch hydrolysis.
3. Identification of *Staphylococcus aureus:* by using the following test
Gram stain, catalase, oxidase, growth on Mannitol salt agar, VJ, and blood agar and Coaulase test.
4. detection of Bacteriocin production:
In this study, the detection of Bacteriocin production was carried out by applying " Reverse agar technic " suggest by (Schwable, 2007).

Procedure

1. Inoculate the petri dish (blood agar) with the culture of (producer strain) *Bacillus subtilis* (a straight-line streak)
2. Inoculate the plate at the optimal temperature for the growth of the Bactria (at 37Cº for 24 hours)
3. A sterile spatula was used to remove the agar from the plate and place it on the cover.
4. Streak a pure culture of the indicator strain (*s. aureus* and *E. coli*) with a line perpendicular to the producer strain on the other side of the agar
5. Inoculate for 24 hours and observe for inhibition of growth on the intersect of cultures (Koneman, 2006)

Results

Identification of Bacteria:

Results of the Identification test of *B. subtilis* and *S. aureus* are shown in tables (1) and (2).

Table 1. Biochemical test of *Bacillus subtilis*

Name of the test	Results
Gram staining	Positive
Catalase	Positive
Oxidase	Variable
Urease	Negative
Spore	Positive
Starch hydrolysis	Positive

Table 2. Biochemical test of *Staphylococcus aureus*

Name of the test	Results
Gram staining	Positive
Catalase	Positive
Oxidase	Negative
Urease	Positive
Coagulase	Positive
Mannitol	Yellow colonies (sugar fermentation)
VJ agar	Black colonies

In addition to figures (10) demonstrate the positive results optioned for each bacteria.

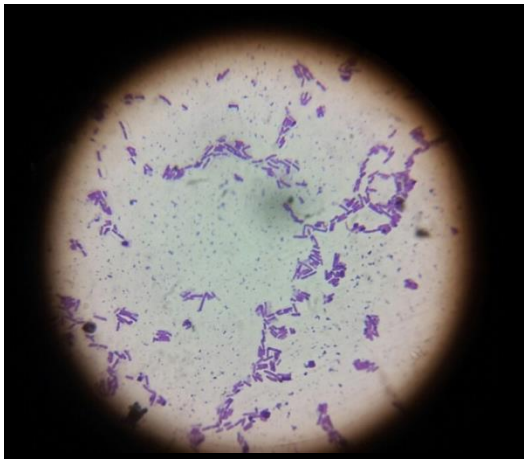

Figure 1. Gram stain of *Bacillus subtilis*

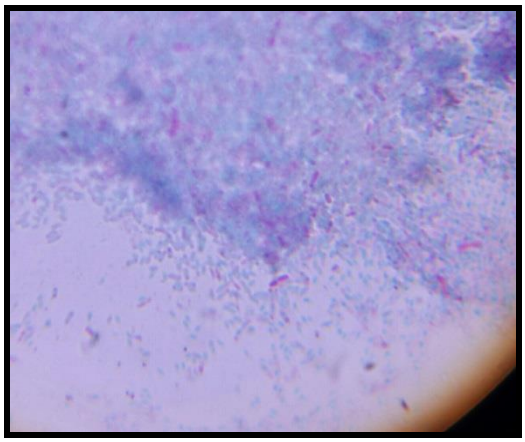

Figure 2. Spore stain of Bacillus subtilis

Figure 3. Catalase test of Bacillus subtilis

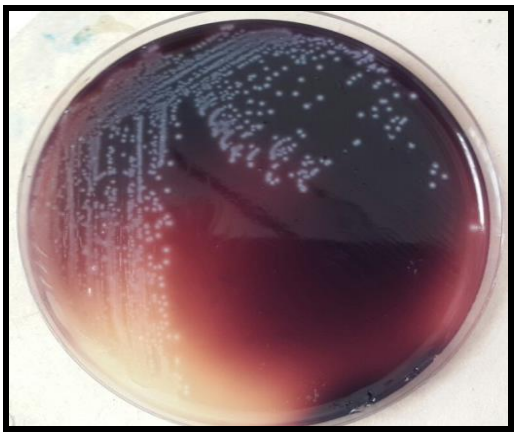

Figure 4. Starch hydrolysis of Bacillus subtilis

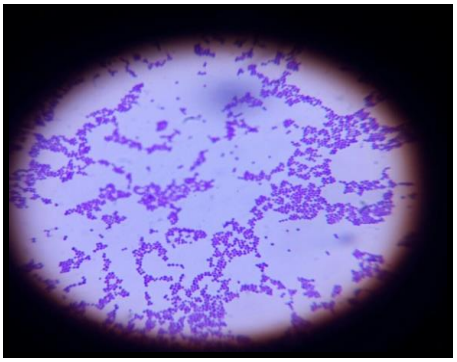

Figure 5. Gram stain of Staphylococcus aureus

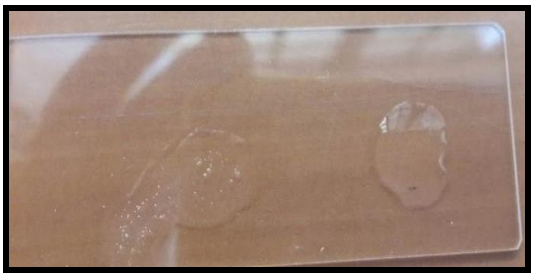

Figure 6. Catalase test of Staphylococcus aureus

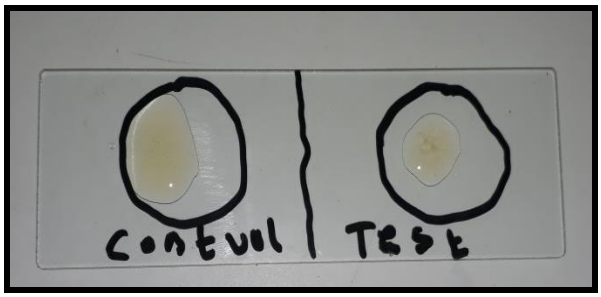

Figure 7. Coagulase test of Staphylococcus aureus

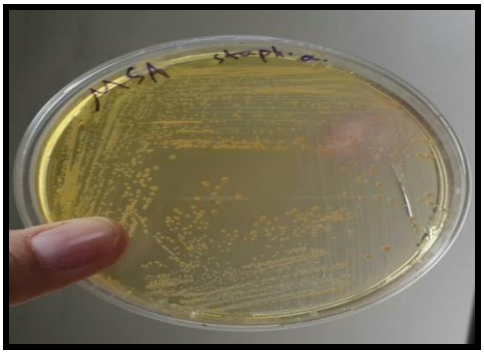

Figure 8. Staphylococcus aureus on Manntiol salt Agar

Detection of Bacteriocin Production:

It was shown that *B. subtilis* is a potent Bacteriocin producer that inhibited *S. aureus* but did not affect the indication strain of *E. Coli*.

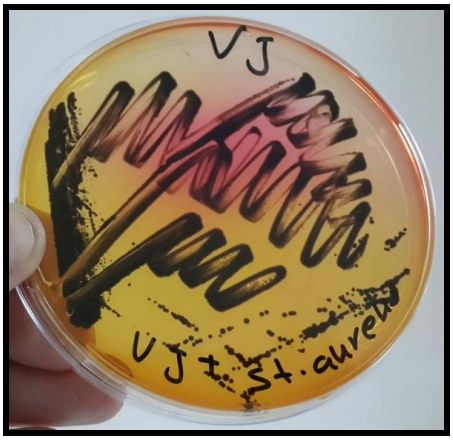

Figure 9. Staphylococcus aureus on V.J.Agar

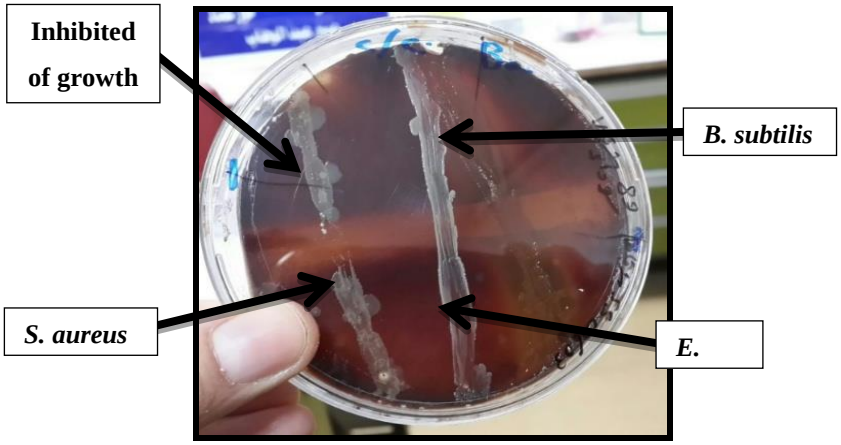

Figure 10. Reverser Agar technic in blood agar

Conclusion

The "reverser Agar Technic" prevents phage particles from reaching the indicator bacteria because there is no direct contact between the producer and the strains being tested. As a result, no inhibition zone due to phages can form, as is often the case with other methods where it is difficult to determine whether a Zone of confluent lysis is caused by phages or bacteriocins.

Resistance to antibiotics is on the rise, posing a therapeutic challenge to the medical community. New approaches and treatment options for infections are therefore becoming increasingly important. Some pathogens would benefit from Bacteriocin instead of the currently prescribed antibiotics. Antibiotic-resistant bacteria like Staphylococcus spp. showed pediculate's resistance to the most widely used antibiotics. Most Bacteriocins have a narrow therapeutic range.

An activator of microorganisms, researchers, on the other hand, believe that bacteriocins' limited antimicrobial activity could be an asset. One of the drawbacks of using throat spectrum antibiotics is that they kill nearly all bacterial species, even those that aren't particularly resistant to ho the one. Because of this, pathogens and commensal bacteria can become resistant to broad-spectrum antibiotics. Due to the relatively narrow spectrum of activity of Bacteriocins, they can be viewed as a specially designed drug that specifically targets a particular pathogen.

Recommendations

Based on our results, the study recommend that Bacteriocic may one day serve as a novel therapeutic agent against pathogens in place of currently used antibiotics.

Acknowledgements or Notes

The authors would like to thank the Iraqi Ministry of Higher Education and the Biology department, University of Mosul for supporting this research .

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the author.

References

Abriouel, H., Franz, C. M., Omar, N. B., & Gálvez, A. (2011). Diversity and applications of Bacillus bacteriocins. *FEMS Microbiology Reviews*, *35*(1), 201-232.

Agarwal, S., Sharma, G., Dang, S., Gupta, S., & Gabrani, R. (2016). Antimicrobial peptides as anti-infectives against Staphylococcus epidermidis. *Medical Principles and Practice*, *25*(4), 301-308.

Al-Dahbi, A. M., & Al-Mathkhury, H. J. (2013). Distribution of methicillin resistant Staphylococcus aureus in Iraqi patients and healthcare workers. *Iraqi Journal of Science*, *54*(2), 293-300.

Bierbaum, G., & Sahl, H. G. (2009). Lantibiotics: mode of action, biosynthesis and bioengineering. *Current Pharmaceutical Biotechnology*, *10*(1), 2-18.

Boyce, J. M., Cookson, B., Christiansen, K., Hori, S., VuopioVarkila, J., Kocagaz, S. Oztop, A. Y., Vandenbroucke-Grauls, C.M.J .E., Harbarth, S., and Pittet, D. (2005). Meticillin-resistant S. aureus. *The Lan. Infect. Disea*, *5*(10), 653-663

Chopra, L., Singh, G., Jena, K. K., & Sahoo, D. K. (2015). Sonorensin: a new bacteriocin with potential of an anti-biofilm agent and a food biopreservative. *Scientific Reports*, *5*(1), 1-13.

Cintas, L. M., Rodriguez, J. M., Fernandez, M. F., Sletten, K., Nes, I. F., Hernandez, P. E., & Holo, H. (1995). Isolation and characterization of pediocin L50, a new bacteriocin from Pediococcus acidilactici with a broad inhibitory spectrum. *Applied and Environmental Microbiology*, *61*(7), 2643-2648.

Cleveland, J., Montville, T. J., Nes, I. F., & Chikindas, M. L. (2001). Bacteriocins: safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology*, *71*(1), 1-20.

Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: developing innate immunity for food. *Nat. R . Microbiol.*, *3*(10), 777-788.

Cotter, P. D., Ross, R. P., & Hill, C. (2013). Bacteriocins—a viable alternative to antibiotics?. *Nature Reviews Microbiology*, *11*(2), 95-105.

Croft, A. C., D'Antoni, A. V., & Terzulli, S. L. (2007). Update on the antibacterial resistance crisis. *Medical Science Monitor*, *13*(6), 103-118.

Dubnau, D. (1982). The *molecular biology, of the bacilli, volume i: bacillus subtilis.* The Public Health Research.

Deurenberg, R. H., & Stobberingh, E. E. (2008). The evolution of Staphylococcus aureus. *Infection, Genetics and Evolution*, *8*(6), 747-763.

Hammami, R., Fernandez, B., Lacroix, C., & Fliss, I. (2013). Anti-infective properties of bacteriocins: an update. *Cellular and Molecular Life Sciences*, *70*(16), 2947-2967.

Hashemizadeh, Z., Bazargani, A., & Davarpanah, M. A. (2011). Blood culture contamination in a neonatal intensive care unit in Shiraz, Southwest-Central Iran. *Medical Principles and Practice*, *20*(2), 133-136.

Kareem, S. M., Al-Jubori, S. S., & Ali, M. R. (2015). Prevalence of erm genes among methicillin resistant Staphylococcus aureus MRSA Iraqi isolates. *Int J Curr Microbiol Appl Sci*, *4*(5), 575-585.

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C., & Winn, W. C. (1997). Diagnostic microbiology. In *The nonfermentative gram-negative* bacilli (pp. 253-320). Lippincott-Raven Publishers.

Mulvey, M. R., & Simor, A. E. (2009). Antimicrobial resistance in hospitals: how concerned should we be?. *Cmaj*, *180*(4), 408-415.

Nicholson, W. L. (2002). Roles of Bacillus endospores in the environment. *Cellular and Molecular Life Sciences CMLS*, *59*(3), 410-416.

Nicolle, P., & Prunet, J. (1964). La propriete colicinogene dans lespece salmonella typhi. In *Annales De L Institut Pasteur*. Masson Editeur.

O'sullivan, L., Ross, R. P., & Hill, C. (2002). Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. *Biochimie*, *84*(5-6), 593-604.

Schwalbe, R., Steele-Moore, L., & Goodwin, A. C. (Eds.). (2007). *Antimicrobial susceptibility testing protocols*. Crc Press.

Snyder, A. B., & Worobo, R. W. (2014). Chemical and genetic characterization of bacteriocins: antimicrobial peptides for food safety. *Journal of the Science of Food and Agriculture*, *94*(1), 28-44.

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To cite this article:

Agha, Y.Y. (2021). Inhibition of the growth of *staphylococcus aureus* by using some antibiotics produced from *bacillus subtilis*. *The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 16*, 231-239.